Nicotinamide Riboside for the Prevention and Treatment of Doxorubicin Cardiomyopathy. Opportunities and Prospects.

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Research Article

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Abstract

Despite the progress in the development of new anticancer strategies, cancer is rapidly spreading around the world and remains one of the most common diseases. At the same time, oncological diseases are detected in patients with late stages of the course in the overwhelming majority of cases. This fact necessitates chemotherapy both as part of a combination and in the view of an independent form of treatment. For more than 40 years, doxorubicin has been widely used in the treatment of solid and hematological tumors. At the same time, the problem of its cardiotoxicity remains unresolved, despite the high efficiency of this drug. Symptomatic therapy is used as a treatment for side-effects of doxorubicin or pathological conditions that have already appeared on their background. To date, there are no treatment methods for doxorubicin cardiomyopathy as such. A drug such as nicotinamide riboside can play an important role in solving this problem. Nicotinamide riboside is a pyridine nucleoside similar to vitamin B3 that acts as a precursor to NAD⁺. There are no such works in cardiomyopathy, despite the abundance of works devoted to the mechanisms of realization of the effects of nicotinamide riboside in various pathologies. The review analyzes information about the effects of NR on various experimental models of pathologies, its role in the synthesis of NAD⁺, and also considers the possibility and prospects of its use for the prevention of doxorubicin cardiomyopathy.

Introduction

According to the World Health Organization, cancer is one of the leading causes of death worldwide. Thereby, almost 10 million people died from this disease in 2020 [1, 2]. Despite the progress in the development of new anticancer strategies, cancer is rapidly spreading around the world and remains one of the most common diseases. At the same time, oncological diseases are detected in patients with late stages of the course in the overwhelming majority of cases. This fact necessitates chemotherapy both as part of a combination and in the view of an independent form of treatment.

For more than 40 years, doxorubicin has been widely used in the treatment of solid and hematological tumors (lymphoblastic leukemia, soft tissue sarcoma, osteosarcoma, breast cancer, thyroid cancer, Wilms tumor, neuroblastoma, bladder cancer, stomach cancer, ovarian cancer, lymphogranulomatosis, non-Hodgkin's disease lymphomas, trophoblastic tumors, refractory ovarian cancer). At the same time, the problem of its cardiotoxicity remains unresolved, despite the high efficiency of this drug, which ensures the survival of patients. Cardiotoxicity is dose-dependent and can develop during or immediately after the administration of doxorubicin, as well as some time after the end of treatment. Thus, acute and chronic doxorubicin cardiotoxicity is separated.

The mechanisms of anthracycline cardiotoxicity are still being investigated in various experimental models [3]. Anthracyclines easily get into cells, localize in the nucleus and intercalate in DNA. Forming a stable ternary complex anthracycline-DNA-topoisomerase 2 β , drugs "poison" the enzyme and prevent the re-ligation of double-stranded DNA breaks. Therefore, anthracyclines initiate programmed cell death [4, 5]. In addition, doxorubicin-induced cardiomyopathy is closely associated with an increase in oxidative stress, as evidenced by reactive oxygen species (ROS)-induced damage such as lipid peroxidation, and decreased levels of antioxidants and sulfhydryl groups. The ability of quinone to convert to semiquinone produces free radicals that actively react with oxygen to form superoxides, hydroxyl radicals, and peroxides. Myofibrillar destruction and dysregulation of intracellular calcium are also important mechanisms, usually associated with doxorubicin-induced cardiotoxicity. Doxorubicin-induced apoptosis targets not only cardiomyocytes, but also endothelial cells, as indicated by the activation of caspase and the degradation of the internucleosomal DNA [6, 7].

To date, there are no treatment methods for doxorubicin cardiomyopathy as such. Often, symptomatic therapy is used as a treatment for side-effects or pathological conditions that have already appeared on their background, which provides a short-term effect. Therefore, the development of approaches aimed at preventing doxorubicin cardiomyopathy remains relevant. In solving this problem, a drug such as nicotinamide riboside can play an important role, which has a number of interesting properties.

Nicotinamide riboside (NR) is a pyridine nucleoside similar to vitamin B3 that acts as a precursor to nicotinamide adenine dinucleotide (NAD⁺). The exceptional importance of NAD⁺ as a coenzyme is due to its enormous need for cellular redox reactions, including most catabolic and anabolic reactions, such as glycolysis, fatty acid oxidation, tricarboxylic acid cycle (TCA), synthesis of fatty acids, cholesterol, steroids, etc. Moreover, depletion of NAD⁺ level is promoted by enzymes that consume NAD⁺, such as sirtuins, poly-ADP-ribose polymerases (PARPs), cADP-ribosyltransferases (CD38/157 ectoenzymes) and mono-ADP-ribose transferases (ARTs), TIR motif-containing 1 (SARM1) [8, 9].

The presented data on the biochemical aspects of NR are the basis for it to be considered as a pathogenetically valid therapeutic agent for the prevention or treatment of doxorubicin cardiomyopathy. Despite the abundance of works devoted to the mechanisms of realization of the effects of NR in various pathologies, there are no such works in cardiomyopathy. This review aims to fill this gap. The article analyzes information about the effects of NR on various experimental models of pathologies, its role in the synthesis of NAD⁺, and also considers the possibility and prospects of its use for the prevention of doxorubicin cardiomyopathy.

Article search and selection strategy

The search for published articles and reviews in peer-reviewed open access journals was carried out using the following databases: PubMed, Google Scholar. In addition, we used existing abstracts of articles or entire articles on ResearchGate without open access. Most of the peer-reviewed articles have been published within the past 15–20 years. Older work was seen rather as a source of fundamental discoveries. Additional databases were also searched through Google using the following keywords: nicotinamide riboside, nicotinamide riboside bioavailability, nicotinamide riboside safety, NAD⁺ metabolism, NAD⁺ and Sirtuins, and similar.

Known pathways of the synthesis of NAD⁺. Feature of NR

Bieganowski and Brenner in 2004 first described the direct contribution of NR to the metabolism of NAD⁺ [10]. In this study, authors characterized NR kinases (NRKs), enzymes that are capable of converting NR directly to NMN (nicotinamide mononucleotide), bypassing NAMPT (nicotinamide phosphoribosyltransferase) in the Salvage pathway of NAD⁺ synthesis. Since the reaction catalyzed by NAMPT limits the rate, it requires the use of energy-consuming PRPP (5-phosphoribosyl 1-pyrophosphate) and must be inhibited by NAD⁺. NR is also able to increase the concentration of NAD⁺ beyond what is achieved through normal metabolism of vitamin B [11, 8].

It is important to note that due to the central role of NAD⁺ in cellular bioenergetics and the maintenance of relatively high concentrations of NAD⁺ metabolites in cells (usually 200–500 μM in mammalian cells), several different pathways are involved in the biosynthesis of NAD⁺. In humans, it includes the eight-step *De novo* pathway from the amino acid tryptophan precursor and additional three and two-step pathways from various nicotinoyl precursors such as nicotinic acid (NA), nicotinamide (NAM), as well as nucleosides, NR, and nicotinic acid riboside (NAR). In general, NAD⁺ metabolism can be divided into four main categories: *De Novo* synthesis; *the Preiss-Handler pathway*, *Salvage pathway* from the corresponding precursors: NA, NR and NAR; *Core Recycling Pathway* via Nicotinamide; ADPR-transfer / NAD hydrolysis, which occurs via various enzymatic pathways leading to the cleavage of the N-glycosidic bond of nicotinamide with the ribose ring, thereby releasing nicotinamide and providing the ADPR nucleophile product [12, 13, 14]. In mammals, the most common precursor is NAM, which can then be used to generate NMN by the rate-limiting enzyme NAMPT [15]. Finally, NMN is converted to NAD⁺ by NMN/NaMN adenylyltransferases (NMNAT). It was found that, the expression of NAMPT and, accordingly, the content of NAD⁺ decreases in many tissues, depending on the aging process, overeating, stress or inflammatory factors of various origins [16]. In this regard, an important role is played by the fact that the maintenance of NAD⁺ levels depends on different biosynthetic pathways and precursors in different tissues [17, 18, 19, 20]. Nevertheless, a

decrease in the expression of the NAMPT enzyme is one of the main reasons for a pronounced decrease in the NAD⁺ level (Fig. 1).

Fig. 1 NAD⁺ biosynthetic pathways. *De novo* biosynthesis begins with the conversion of tryptophan (Trp) to *N*-L-formylkynurenine either by indolamine-2,3-dioxygenase (IDO) or tryptophan-2,3-dioxygenase (TDO). After four reaction steps, *N*-L-formylkynurenine can subsequently be converted to the unstable 2-amino-3-carboxymuconate-6-semialdehyde acid, which can undergo non-enzymatic cyclization to quinolinic acid. The last step of *de novo* biosynthesis consists of the quinolinate-catalyzed phosphoribosyltransferase (QPRT) formation of NA mononucleotide (NaMN) using PRPP (5-phosphoribosyl 1-pyrophosphate). *The Preiss-Handler pathway* is initiated by NA phosphoribosyltransferase (NAPRT) to form NaMN. Further, NaMN is converted into adenine dinucleotide NA (NAAD) by the enzymes NMN adenylyltransferase (NMNAT1-3) together with ATP. Finally, NAAD is converted to NAD⁺ via an amidation reaction catalyzed by the enzyme NAD⁺ synthase (NADSYN). *Core Recycling* and *Salvage Pathways* are the shortest ways to synthesize NAD⁺ from NAM and NR (2 steps). NAM is converted by the rate-limiting nicotinamide phosphoribosyltransferase (NAMPT) to form NMN using PRPP as a cosubstrate. NMN is also a product of NR phosphorylation by NR kinases (NRK1-2). The subsequent conversion of NMN to NAD⁺ is catalyzed by the NMNAT1-3 enzymes. Further, the synthesized NAD⁺ is used in the work of such enzymes as sirtuins, PARPs, CD38 / 157. NR, nicotinamide riboside; NAM, nicotinamide; NMN, NAM mononucleotide; AFMID, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; KYNU, tryptophan 2,3-dioxygenase; 3HAO, 3-hydroxyanthranilic acid oxygenase

The need for NAMPT can be circumvented by direct conversion of NR to NMN by two nicotinamide ribosokinases, NRK1 and NRK2 [21]. Therefore, we will pay special attention to the synthesis of NAD⁺ from nucleosides (NR and NAR). NRK1 and NRK2 are encoded by the human genome and by the genomes of other mammalian organisms (encoded by the *Nmrk1* and *Nmrk2* genes, respectively). X-ray crystallographic, biochemical data show that these enzymes exhibit high affinity for NR and NAR, but their substrate specificity is different. NRK1 can use GTP (guanosine triphosphate) as an additional substrate for NR phosphorylation, while NRK2 is restricted to ATP [21, 22]. Synthesis of NMN with NRK avoids the need for energy-intensive PRPP. Additionally, NR can be converted to NAM by purine nucleoside phosphorylase (NP), which is subsequently converted to NAD⁺ via NMN synthesis by NMNAT [23, 24, 19]. NR and NAR increase NAD⁺ levels dose-dependently and up to 2.7-fold in a single 1000mg dose in mammalian cells, in contrast to nicotinamide or nicotinic acid at the same concentrations [25, 23, 26]. Kulikova et al. in 2015 showed that NR can be produced in mammalian cells and released extracellularly, suggesting possible intercellular metabolic networks involving the creation, release and transport of NR (and NAR) to other cells [27].

The effectiveness of NR in increasing cellular NAD⁺ has led to studies questioning whether it can treat diseases such as cardiovascular complications of various origins, neurodegenerative disorders or metabolic syndromes, based on the idea that a decrease in NAD⁺ levels may be a risk factor in these states.

Metabolism NAD⁺

The importance of NAD⁺ is reflected in its balance in various cellular compartments, and this balance is manifested in the activity of enzymes that consume NAD⁺ (sirtuins, PARPs, CD38/157, SARM1), stress and aging mediators, the production of which is enhanced by factors such as DNA damage, oxidative stress and inflammation. The sirtuin family (NAD⁺-dependent deacetylases / deacylases) includes seven genes and the corresponding proteins encoded by them with different localization within the cell, enzymatic activity and targets (mitochondrial SIRT3-5; nuclear SIRT1,6,7; cytoplasmic SIRT2; SIRT1 is able to move between the nucleus and the cytoplasm) [28, 29]. Sirtuins are conservative regulators of aging and longevity in various organisms and ones are considered major metabolic switches due to their many regulatory functions in metabolism, DNA repair, stress response, chromatin remodeling, and circadian rhythm. SIRT1 and SIRT2 are believed to be responsible for most of the NAD⁺ consumption under normal conditions. Increased NAD⁺ levels strongly

correlate with sirtuin activation during fasting and calorie restriction [30]. The enzymatic mechanisms of sirtuins in the regulation of cellular metabolism are still being actively studied. At this stage, it is known that the enzymatic activity of sirtuins includes the removal of the acetyl group from the lysine residues of the target proteins in a two-step process: firstly, NAD^+ is hydrolyzed to NAM and ADP-ribose. Then, the acetyl group of the target protein is cleaved and transferred to ADP-ribose, providing the formation of an intermediate peptidyl-ADP-ribose. Acetyl-ADP-ribose is subsequently released. Previously, it was impossible to investigate changes in NAD^+ levels and sirtuin activity due to technical difficulties. It is now known that nuclear SIRT1, SIRT6 and SIRT7 are critical regulators of DNA repair and genome stability; mitochondrial SIRT3, SIRT4, SIRT5 and nuclear SIRT1 regulate mitochondrial homeostasis and metabolism. SIRT1 also plays a significant role in the turnover of defective mitochondria, thus being a key factor in maintaining the quality of mitochondrial metabolism [31, 32, 33, 34]. Canto in 2012 described a number of transcription factors that can regulate the expression of SIRT1 under starvation conditions: CREB, PPARs, FOXOs, and p53. ChREBP transcription factors activated by high glucose uptake, conversely, suppress SIRT1 levels [35]. In turn, SIRT2 targets key metabolic regulators such as FOXO, the p65 NF- κ B subunit, and phosphoenolpyruvate carboxykinase (PEPCK) [36, 37, 30], suggesting its role in the regulation of inflammation, gluconeogenesis, and the response to caloric restriction. SIRT3 target proteins include mitochondrial respiratory complexes, TCA cycle proteins, and enzymes associated with lipid metabolism and detoxification of reactive oxygen intermediates such as isocitrate dehydrogenase (IDH) and superoxide dismutase (SOD). PGC-1 α , as the main organizer of mitochondrial biogenesis, positively regulates SIRT3 at the transcriptional level, in response to various energy stresses, starvation [38]. SIRT4,5 are currently less studied. But SIRT4 is known to act as a modulator of fat metabolism in hepatocytes and myocytes. SIRT4 contributes to the opposite effects of SIRT1 on insulin secretion [39], and SIRT3 contributes to the opposite effects of SIRT1 on fat oxidation [40]. Du in 2011 discovered that the main function of SIRT5 is not as a deacetylase, but as demalonizase and desuccinylase [41]. SIRT6 is attracting researchers for its role in genomic DNA stability, metabolism, and aging. Its pronounced expression provides protection against obesity associated with a high-fat diet, and SIRT6 is also able to work as a co-repressor of HIF-1 α [42]. SIRT6 knockout mice demonstrate severe defects such as lymphopenia, loss of subcutaneous fat, decreased bone mineral density, hypoglycemia, and decreased levels of insulin-like growth factor (IGF) -1. SIRT7 is localized in the nucleolus and has been described as a component of the transcriptional apparatus of RNA polymerase I (Pol I) [43]. There is also evidence of a possible role for SIRT7 in cancer development, but further study is required [44]. In general, we could say that at this stage of the development of science, sirtuins have firmly established and become central participants in understanding how NAD^+ levels affect cellular homeostasis and how exactly in the future they may be used in a therapeutic direction.

The PARP family of proteins also use NAD^+ in large quantities in their work together with sirtuins. The human PARP family includes 17 proteins characterized by poly (ADP-ribosyl) polymerase activity or mono (ADP-ribosyl) polymerase activity. Ones catalyze the reaction of transfer of ADP-ribosyl (adenosine diphosphate-ribose residue) to a poly-ADP-ribosyl chain bound to a protein, in which the donor of ADP-ribose is NAD^+ . It is known that only PARP1, PARP2, and PARP3 are localized in the nucleus, respond to DNA breaks, and contribute to the DNA repair process [45]. PARP activation increases as DNA damage accumulates over time, which, in turn, SIRT1 activity decreases due to both substrate competition, being in the same cellular compartment, that is, in the nucleus. It is also important to note that PARP1 has higher binding affinity and faster kinetics for NAD^+ compared to SIRT1 [29]. To date, PARP1 is the best characterized of all members of the family. This molecule is widely associated not only with the aging process due to its high activity of NAD^+ consumption during DNA repair, but also with other normal and pathophysiological processes, confirming its key role in maintaining homeostasis in the cell. For example, there is a strong correlation between PARP activation, decreased SIRT1 activity, and decreased NAD^+ levels in patients with group A xeroderma pigmentosa, ataxia, telangiectasia, and Cockayne's syndrome. When treating Cocaine mice with NAD^+ precursor supplementation, has been shown to increase lifespan and reduce severe phenotypic manifestations caused by PARP1 hyperactivation, providing strong evidence that the negative consequences of PARP1 activation are mediated by dysregulation of NAD^+ homeostasis in response to extensive DNA damage and genotoxic stress [46]. As for the rest of the members of the PARP family, PARP2 and PARP3 are structurally related to

PARP1; ones have a similar catalytic domain required for the regulation of DNA repair and transcription [47, 48]. The functions and effects of PARP4-7 have not yet been definitively determined on metabolism of NAD^+ .

In addition, ectoenzymes CD38 / 157 with glycohydrolase and ADP-ribosyl cyclase activity use NAD^+ for the production of cADP-ribose and NAAD(P) (nicotinic acid adenine dinucleotide (phosphate)). In turn, cADP-ribose and NAAD(P) are secondary messengers that promote the mobilization of Ca^{2+} [49]. This fact determines the understanding of the role of CD38 / 157 in modulation of many cellular processes: survival, metabolism, activation of immune cells, also in the biology of aging, for example, age-related diseases (rheumatoid arthritis, cancer) [50]. CD38 can also degrade NAD^+ , NR and NMN intermediates, which further reduces NAD^+ . The effect of CD38 on NAD^+ content has been demonstrated in CD38-deficient mice in which NAD^+ levels remain high [51]. This preserves mitochondrial respiration and metabolic function with age. Moreover, inhibition of CD38 can increase NAD^+ levels and improve glucose and lipid metabolism [51]. Despite CD38 and CD157 are members of the same enzymatic family and are genetically homologous, they are structurally and localized differently. CD38 is a type II or type III transmembrane protein, first described in the late 1970s as a marker of T cell activation. CD38 is now known to be ubiquitous, especially during inflammation [53]. CD157 is a glycoposphatidylinositol-anchored protein that was first identified in the myeloid compartment of the hematopoietic system. One is also expressed by other cells, including B-cell progenitors, Paneth cells, and endothelial cells in the intestine, pancreas, and kidneys [54]. In addition to enzymatic function, CD38 and CD157 also work as cellular receptors. Deaglio et al indicate a role for CD38 as an adhesion receptor that interacts with CD31 to mediate the transport of immune cells and their movement through the endothelium. Thus, it activates the proliferative response in lymphocytes of chronic lymphocytic leukemia, confirming the detrimental role of CD38 in blood cancer [55, 56]. Still CD157 as a receptor remains poorly understood.

SARM1 is the new enzyme involved in the metabolic reactions of NAD^+ . SARM1 is an enzyme that is the most evolutionarily conserved member of the Toll-interleukin receptor (TIR) family. SARM1 is able to hydrolyze NAD^+ into cADPR and therefore functions as a Ca^{2+} signaling enzyme similarly, to CD38, but SARM1 increases cADPR much more efficiently than CD38. It also plays a key role in the degeneration of axons after damage. It is known to be more expressed in neurons and promotes neuronal morphogenesis and inflammation [57]. SARM1 triggers an axonal destruction program that catalyzes the production of nicotinamide and ADPR / cADPR from NAD^+ , causing bioenergetic depletion of NAD^+ and ATP in response to neuronal damage. This is followed by the activation of the calpain and the final dismantling of the axon [58]. Additionally, SARM may play a role in mitophagy and possibly other as yet unknown cellular functions, but its main known function in mammals today is to mediate neuronal cell death [59]. Key points of NAD^+ metabolism are presented in **Fig. 2**.

Fig. 2 The balance of NAD^+ is the balance of synthesis, consumption and recirculation in various subcellular compartments (cytoplasm, nucleus and mitochondria). After entering the cell, NAD^+ precursors are metabolized by four main pathways (Fig. 1) to NAD^+ . In the cytosol, nicotinamide (NAM) is converted to nicotinamide mononucleotide (NMN) by the intracellular form of NAM phosphoribosyltransferase (iNAMPT). NMN is then converted to NAD^+ by NMN transferase 2 (NMNAT2) bound to the outer Golgi membrane in the cytoplasm. NAD^+ is converted to NADH / NADPH during the redox cycle, is also used during aerobic / anaerobic glycolysis and is consumed by NAD-dependent enzymes (SIRT2, PARP1-3, CD38 / 157, SARM1). In mitochondria, NMN is converted to NAD^+ by NMNAT3. NAD^+ is used by the TCA cycle to generate ATP and is additionally used by mitochondrial sirtuins 3-5 (SIRT3-5) and PARP1, which generate NAM. Studies show the presence of NAD^+ , NADH and NMN transporters in the mitochondrial membrane, but no specific transporters have yet been identified. It is still not clear whether NAM can be converted back to NMN within the mitochondria, or whether it is transported / diffused from the mitochondria into the cytosol. Inside the nucleus, NMN is converted to NAD^+ by NMNAT1, while NAD^+ is consumed here mainly by SIRT1,6,7, PARP1-3, SARM1. As in the cytosol, NAM is returned back to the NMN by iNAMPT

The role of NAD⁺ as a coenzyme in most metabolic pathways suggests that NAD⁺ limitations affect metabolic efficiency and therefore NAD⁺ levels may change during various physiological processes. This is confirmed by a number of studies on worms, rodents and human cell models [60, 61, 62]. For instance, a decrease in NAD⁺ content in muscle progenitor cells leads to a SIRT1-mediated metabolic switch that induces premature differentiation and loss of regenerative capacity, reflecting a phenotype typical of aging muscles. The link between metabolism and NAD⁺ is further supported by the observation that tissue NAD⁺ levels are reduced with diets high in fat. In contrast, NAD⁺ increases in response to exercise or calorie restriction [63, 32, 64]. The addition of NAD⁺ precursors has been shown to increase the lifespan of budding yeast and worms. In the studies of Khan et al and Cerutti et al in 2014 in mammals, an increase in NAD⁺ levels was associated with an improvement in mitochondrial function under stress conditions, which in turn leads to protection against metabolic complications of various origins [65, 66]. It is also important to note that liver NAD⁺ levels are dynamically altered by circadian rhythms. The heterodimeric complex of the main factors of circadian transcription BMAL1 and CLOCK controls the expression of the *Nampt* gene encoding the NAMPT enzyme. The activation of circadian transcription factors decreases under the influence of various inflammatory cytokines, oxidative stress; therefore, the synthesis of NAD⁺ is impaired. They are controlled by SIRT1 according to the principle of feedback, it also regulates the expression of the *Bmal1* and *Clock* genes in the suprachiasmatic nucleus through the RORα and PGC-1α complex [67, 68]. This important aspect also demonstrates the ability of NAD⁺ to dynamically respond to various physiological stimuli.

Since glycolysis in the cytoplasm and the TCA cycle in mitochondria are able to influence metabolic homeostasis by changing the cytosolic and nuclear levels of NAD⁺ / NADH, the concentration of NAD⁺ is always limited. After any DNA damage, NAD⁺ levels can drop so low that glycolysis and the flow of substrate into mitochondria are blocked, eventually leading to cell death. This fact emphasizes the need to understand the mechanisms of NAD⁺ metabolism, the relationship of its precursors, since their homeostasis and interaction are important for maintaining cell viability and ATP levels. It is also important to understand how exactly it can be used as a possible therapeutic effect for the prevention of various pathologies / complications, for example, doxorubicin cardiomyopathy.

Effects of NR in small laboratory animal models

The study of Canto et al. in 2012 is the first major study of the effect of NR on the metabolic state of C57Bl / 6J mice following a high-fat diet. In Canto's study, mice fed 400 mg / kg / day of NR in the diet were protected from weight gain, were more sensitive to insulin, and had increased mitochondrial content in skeletal muscle and brown adipose tissue compared to untreated controls [69].

The study in 2014 by Kevin D. Brown showed that a 1000mg / kg NR injection given to mice twice daily for 5 days prevented noise-induced hearing loss (NIHL) and spiral ganglion neurite degeneration, even after exposure to noise [70]. Brown demonstrated that these effects are mediated by a NAD⁺-dependent mitochondrial sirtuin, SIRT3. Since mice overexpressing SIRT3 are resistant to NIHL; deletion of SIRT3 reverses the protective effects of NR and the expression of biosynthetic NAD⁺ enzymes. These data indicate that NR administration activates the NAD⁺-SIRT3 pathway, which reduces noise-induced neurite degeneration. NR has a therapeutic effect in various muscle pathologies. For instance, NR improves mitochondrial function, induces autophagy in mitochondrial myopathies, and decreases the mitochondrial unfolded protein response in a model of heart failure caused by cardiospecific transferrin receptor deletion [65]. Cerutti et al. used a diet (400mg / kg HP) for four weeks. Frederick et al. in 2016 showed that injection of NR to both female and male mice eliminates progressive wasting syndrome in the Duchenne muscular dystrophy model and restores animal endurance in one week of treatment by adding 400 mg / kg NR to drinking water [71] NR generally increases lifespan and is thought to be based on improved stem cell function [71]. Gong et al. demonstrated dramatic improvement in Alzheimer's disease in a Tg2576 mouse model with a diet of 250 mg / kg NR in a 2013 study [73, 8].

The studies of Trammell et al. are of particular interest. He determined the dose-dependent effects of NR on the metabolism of NAD⁺ in human blood [24, 74, 75, 76]. Authors showed that a single oral dose of 1000 mg of NR in humans can increase blood NAD⁺ levels by 2.7 times and that oral administration of NR increases liver NAD⁺ levels in mice with excellent pharmacokinetics superior to those of nicotinic acid and nicotinamide. Separately, Trammell investigated single doses of 100, 300, 1000 mg NR in humans. He also worked with C57Bl / 6J mice and injected intraperitoneally (IP) 500 mg / kg NR for 6 days.

NR administration prior to sepsis simulation prevents lung and heart damage and improves survival in mice by inhibiting oxidative stress through NAD⁺ / SIRT1 signaling and HMGB1 (plasma high mobility group box-1) release [77]. Hong et al. in 2018 injected intraperitoneally 100, 300, 500 mg / kg NR into C57Bl / 6J mice with 30 minutes before the injection of feces into the peritoneum. Zheng et al. in 2019 also administered 100, 300, and 500 mg / kg NR in a single dose of IP 30 minutes before the 20 mg / kg doxorubicin injection. His study showed that NR administration increased NAD⁺ levels and decreased heart damage and myocardial dysfunction in chemically-treated mice. Similar protective effects of NR have been replicated in cultured cardiomyocytes after doxorubicin treatment. NR prevents blockage of autophagic flow, accumulation of autolysosomes, and oxidative stress in cardiomyocytes. In general, NR increases the clearance of autolysosomes through NAD⁺ / SIRT1 signaling, thereby preventing doxorubicin cardiotoxicity [78]. There are also studies demonstrating the positive effect of NR on the models of retinal degeneration in BALB / c mice, which were injected with IP 1000 mg / kg of the drug [79].

Based on the existing information to date, it can be concluded that the researchers, since the beginning of the study of NR, a particular precursor of the NAD⁺ metabolism, focus their attention on the introduction of the drug from 100 to 1000 mg / kg. Everyone chooses their dose of NR based on previous work and the needs of their research, using two methods of drug administration: oral and intraperitoneal.

Conze and his colleagues conducted a large study to determine the safety of the synthetic analogue of NR from Niagen™ using the reverse mutagenesis assay of bacteria (Ames assay), the analysis of chromosomal aberrations in vitro, the analysis of micronuclei in vivo and also investigated the toxicity of the drug in male and female Sprague-Dawley rats within 14 and 90 days [80]. Initially, they carried out work on the study of acute toxicity after oral administration of 5000 μ / kg NR. There was no mortality from such the dose. Further, based on the results of a 14-day study with daily administration of NR at 750, 1500, 2500, 5000 mg / kg, work was carried out to study the subchronic toxicity of Niagen, which lasted 90 days. Animals received orally 300, 1000, 3000 mg / kg of the drug. The results of the study demonstrated that NR is not genotoxic and that the toxicity profile of NR is similar to that of nicotinamide at the highest dose tested. The lowest level of side effects was observed at a dose of NR of 1000 mg / kg, and toxicity was completely absent with the introduction of 300 mg / kg. The main target organs were the liver, kidneys, ovaries and testes.

Based on the study of Conze et al. in 2016, a protocol for oral administration of HP was selected in a number of studies by Kourtzidis et al. in 2016 and 2018. He studied the effect of the NAD⁺ precursor on physical performance by daily oral administration of male Wistar rats 300mg / kg NR for 21 days [81,82]. He showed that chronic NR intake increases NADPH levels and dramatically increases liver glycogen but not muscle glycogen, decreases antioxidant enzyme activity, decreases blood glucose levels, and maximizes lactate production during exercise. Kourtzidis indicates that long-term intake of NR can lead to dysregulation of redox and energy metabolism and impairment of physical health in rats. Thus, an exogenously administered drug to healthy people can lead to undesirable side effects in addition to positive ones.

Hamity et al. in 2017 showed in female Sprague-Dawley rats that were orally administered 200 mg / kg NR before intravenous injection of paclitaxel (a model of paclitaxel-induced peripheral neuropathy) and after 24 days, a decrease in the development of tactile sensitivity and dulling of avoidance behavior places [83]. Hamity's results suggest that agents that increase NAD⁺ are a key cofactor for mitochondrial oxidative phosphorylation systems and cellular redox systems,

which involved in energy metabolism. Ones represent a novel therapeutic approach for the relief of chemotherapy-induced peripheral neuropathies.

In 2018, the study was published that used a completely new method of introducing NR [84]. This study investigated the effect of NR by intravenous administration of 50 mg / kg, on the functional state of the endothelium, microcirculation and intestinal morphology in acute mesenteric ischemia and reperfusion. The results showed that NR improves the relaxation function of mesenteric vessels and contributes to the protection of the intestinal wall from ischemia-reperfusion injury.

With regard to the studies done on rats, there are very few of them at the moment. Researchers often use only one route of HP administration, the oral route, and focus their attention on the introduction of 200-300 mg / kg referring to the article by Conze, Crespo-Barreto and Kruger, 2016. **Table 1** summarizes the data on the studies described in this section.

Table 1 Effects of NR on small laboratory animal models

No Reference	Sex, age	Dose of NR	Duration/Administration frequency	Route of administration	Effect
[69]	C57Bl/6J mice, male, 8 weeks	400 mg/kg	12 weeks	diet	enhance oxidative metabolism; protection against high fat diet-induced metabolic abnormalities; improved insulin sensitivity
[73]	Tg2576 mice	250 mg/kg	3 months	diet	benefit cognitive function and synaptic plasticity
[70]	C57Bl/6J mice, male, 8-10 weeks	1000 mg/kg	twice daily for 5 days	IP	activates a NAD ⁺ -SIRT3 pathway; reduces neurite degeneration
[65]	unspecified	400mg/kg	4 weeks	diet	Improved mitochondrial respiratory capacity in muscle
[75]	C57BL/6J mice, male	500mg/kg	6 days	IP	improved glucose tolerance; reduced weight gain, liver damage and the development of hepatic steatosis in prediabetic mice; protect against sensory neuropathy
[24]	C57Bl/6J mice, 12-week-old male; 6–8-week-old	185 mg/kg; 500 mg/kg	1 week; 6 days	diet; IP	the rise in NAAD is a highly sensitive biomarker of effective NAD ⁺ repletion.
[72]	C57BL/10ScSn- Dmd ^{mdx} /J mice, male	400 mg/kg	6–8 weeks	diet	induced the mitochondrial unfolded protein response; delays senescence of neural SCs and melanocyte SCs; increases mouse life span
[77]	C57BL/6 mice, male, 2 months	100, 300, 500 mg/kg	single dose	IP	prevent lung and heart injury; improves the survival in sepsis
[78]	C57BL/6 mice, male, 2 months	100, 300, 500 mg/kg	single dose	IP	elevated NAD ⁺ levels, reduced cardiac injury and myocardial dysfunction
[79]	BALB/c mice, male, 3 months	1000 mg/kg	single dose	IP	protective effects of NR treatment in a mouse model of retinal degeneration
[80]	Sprague- Dawley rats, male and female	5000 mg/kg; 750, 1500, 2500, 5000 mg/kg; 300, 1000, 3000 mg/kg	Single; 14 days; 90 days	gavage	toxicity profile similar to nicotinamide, target organs of toxicity are liver, kidney, ovaries, and testes; the lowest observed adverse effect

					level for NR is 1000 mg/kg/day; the no observed adverse effect level was 300 mg/kg/day
[81]	Wistar rats, male, 4 months	300 mg/kg	21 days	gavage	negative effect of NR administration on physical performance
[82]	Wistar rats, male, 4 months	300 mg/kg	21 days	gavage	increase NADPH levels in liver, but not in muscle, decrease the activity of major antioxidant enzymes in muscle; excessively increase glycogen in liver, but not in muscle; decrease glucose concentrations in blood; decrease maximal lactate production during exercise
[83]	Sprague-Dawley rats, female	200 mg/kg	7 days prior to and 24 days post-paclitaxel; 21 days beginning 14 days post-paclitaxel	gavage	reverse the well-established tactile hypersensitivity in a subset of rats and blunt escape-avoidance behavior
[84]	Wistar rats, male	50 mg/kg	single dose	IV	protect the intestinal wall from ischaemia-reperfusion injury; improving the relaxation function of mesenteric vessels

NR, nicotinamide riboside; IP, intraperitoneal; IV, intravenous

Discussion

NAD⁺ metabolism plays a key role in the regulation of cell life (glycolysis, fermentation, pyruvate dehydrogenase, TCA cycle and oxidative phosphorylation) due to the fact that NAD⁺ levels are important for optimizing metabolic parameters in both normal and pathological conditions. It is important that NAD⁺(H) levels are in a constant balance between synthesis and consumption in various cellular compartments: nucleus, mitochondria and cytoplasm to maintain redox homeostasis. Deficiency of various etiologies leads to redox stress and is accompanied by the development of pathological conditions. An example of this development is the action of anthracycline antibiotics.

To date, as mentioned above, the mechanisms of the damaging effect of doxorubicin on the myocardium and possible ways of preventing them are being actively studied. The mechanisms of action of doxorubicin are based on its intercalation with DNA and inhibition of topoisomerase 2 β . The cardiotoxicity of doxorubicin depends on various signaling mechanisms. Above all, doxorubicin-induced cardiotoxicity is caused by the development of oxidative stress. Doxorubicin undergoes a redox cycle in complex I of the electron transport chain, which leads to massive production of ROS and subsequent damage to DNA, proteins and lipids, ultimately leading to cell dysfunction and cell death.

The balance between the formation of free radicals and antioxidant defense systems is disturbed in diseases associated with oxidative stress. Free radicals in large quantities damage lipids, DNA and proteins, or can react with metal bound to proteins, affecting all vital components of cells and tissues. Various enzyme systems are designed to deal with free radical damage and therefore protect against free radical-induced diseases, among which PARPs and SIRT1s that consume NAD^+ may play a key role. But it is important to understand exactly how. ROS cause DNA damage and therefore activate PARP in an attempt to restore DNA integrity. PARP activation has pleiotropic effects: induction of necrosis, mitochondrial damage, pro-inflammatory actions, reprogramming of gene expression that worsen free radical-mediated pathologies [85]. PARP level and activity is also strongly correlated with mitochondrial activity [45]. For example, long-term PARP activation due to depletion of NAD^+ cell pools lead to a shutdown of mitochondrial function. Conversely, upon inhibition of PARP1 / 2, mitochondrial activity is not only maintained, but further enhanced by activation of SIRT1. In turn, induction of SIRT1 is able to protect against oxidative stress. SIRT1 modifies numerous components of the cell cycle coordination mechanism (e.g., p53 and FOXO) in oxidative damage that results in cell cycle arrest and suppression of apoptosis [86, 87]; induces antioxidant defense systems such as manganese superoxide dismutase (MnSOD); restores mitochondrial biogenesis damaged by oxidative stress [88]; promotes the activation of autophagy, which is impaired by doxorubicin and leads to the accumulation of non-degradable autolysosomes [89].

Therefore, activation of SIRT1 and PARP has opposite characteristics under conditions of oxidative stress. Because PARP activation contributes to additional damage to cells and tissues during oxidative stress. Thus, the study of the PARP-SIRT1 interaction will help to understand their functional role / mechanisms in the metabolism of NAD^+ in order to use them in the future as a therapeutic effect in pathologies of the cardiovascular system, for instance, in the development of doxorubicin cardiomyopathy.

It is important to note that impaired NAD^+ homeostasis due to mitochondrial dysfunction is central to the development of cardiac hypertrophy, heart failure, and cardiomyopathy. Changing the redox capacity of the heart further increases its susceptibility to stress. Furthermore, a transition from fatty acid oxidation and oxidative phosphorylation to other forms of substrate metabolism (glycolysis and oxidation of ketones) often occurs with the development of heart failure and cardiomyopathy, while the NAD^+ / NADH ratio decreases, the NAMPT enzyme is repressed [90]. In this regard, the addition of NR is of particular interest, since one is able to normalize the NAD^+ / NADH ratio in the myocardium, exhibits protective effects against unfavorable cardiac remodeling and, which is important, activates the synthesis of NAD^+ through NRK1 / 2. There is information that the NAMPT enzyme is repressed in some mouse models of cardiac injury, while the expression of NRK2 is greatly increased. A similar shift is observed in humans with cardiomyopathy [91]. Thus, it has been suggested that activation of NAD^+ synthesis via the NRK2 pathway represents a common adaptive mechanism in heart failure, while the *Nrk2* gene may be activated in response to NAMPT inhibition [19]. Moreover, the synthesis of NAD^+ via NRK1 / 2 is a more economical way in terms of the consumption of ATP molecules (1 molecule), while the synthesis using NAMPT requires 3 ATP. An interesting feature of NR is that, the activation of SIRT mechanisms and maintenance of Ca^{2+} homeostasis is stimulated by increasing the production of NAD^+ [92]. In turn, activation of sirtuins is able to protect against cardiac hypertrophy, metabolic dysregulation, and cardiac inflammation. These data confirm the preference for therapeutic use and as a maintenance therapy of NR as a precursor of NAD^+ (Fig. 3).

Fig. 3 Hypothesis of the impact of NR in the development of doxorubicin cardiomyopathy. Today, it is a popular assumption that the development of doxorubicin (DOX) cardiotoxicity occurs due to the massive generation of ROS, which in turn is caused by a secondary mechanism due to the suppression of topoisomerase β . In consequence DNA damage grows, enzymes of its repair, PARPs, CD38 are activated, while the activity of sirtuins and NAMPT (the main pathway of NAD^+ synthesis through NAM under normal physiological conditions, *Core Recycling Pathway*) is suppressed, the work of Complex I in the Electron transport chain is disrupted, using NADH as an electron donor, creating NAD^+ . Damage to Complex 1 and ATP production leads to accumulation of ROS that increase oxidative stress. Separately doxorubicin

reduces tight junction formation by decreasing the expression of the occluded zone (ZO) -1, which can increase doxorubicin levels in the capillary endothelium of the heart muscle. It also reduces the nitric oxide (NO) level by enzymatic inhibition, by reducing the level of endothelin (ET)-1, accumulation of ROS [93]. All of these mechanisms lead to a significant decrease in NAD⁺ levels, the inability of the antioxidant system of the cardiomyocyte to cope with increased oxidative stress, mitochondrial dysfunction, which ultimately leads to apoptosis of endothelial and cardiac cells. The administration of nicotinamide riboside (NR) can significantly increase the level of NAD⁺ through a less energy-intensive synthesis through NRK1 / 2. An increase in the level of NAD⁺ activates the work of sirtuins, which are able to induce antioxidant defense systems that restore mitochondrial biogenesis damaged by oxidative stress and promote the activation of autophagy, which is disturbed by doxorubicin, leading to the accumulation of non-degradable autolysosomes. NMN synthesis via NR inhibits endothelial inflammation and improves NO-dependent function. Activation of endothelial SIRT1 controls endothelial homeostasis and vascular functionality by modulating the activity of endothelial nitric oxide synthase (eNOS), p53, angiotensin II receptor (Ang II), FOXO1 and other mechanisms [94]

Additionally, the question arises about the method of administration of NR to patients undergoing chemotherapy. As indicated in the previous chapters, the drug is often administered orally and the same doses intraperitoneally (not possible in humans). But an interesting possibility is the delivery of NR intravenously [84]. Understanding the fact that people undergoing chemotherapy receive, in addition to treatment, also side effects that affect the work of various organ systems, including the digestive tract (intestinal mucositis) [95, 96]. Therefore, taking the required dose of NR orally may be ineffective due to possible intestinal malabsorption. The study of direct intravenous administration of the drug can be considered as a good alternative in supportive care of the patient.

Considering the damaging effect of doxorubicin is cumulative, progressive, it is also important to consider the frequency of administration of NR to patients undergoing chemotherapy. Studying the characteristics of the time of accumulation and consumption of NAD⁺ during injections of NR, as well as considering the options for its administration regarding the mechanisms of development of anthracycline cardiomyopathy, can play an important therapeutic role in the prevention or treatment of doxorubicin cardiotoxicity.

Thus, the administration of NR shows efficacy in the normalization of several metabolic pathways, such as oxidative stress, inflammatory response, and circadian rhythm. Long-term administration of NR can be a highly effective way to maintain both increased SIRT1 activity in tissues and organs, where NAMPT-mediated NAD⁺ biosynthesis is impaired, and to resist cardiovascular pathologies that developed during chemotherapy, including as a therapy for doxorubicin cardiomyopathy. However, all possible mechanisms of NAD⁺ metabolism and regulation of NAD⁺ mediated proteins are still not clear and require a more qualitative study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Author contributions

E.P. collected supporting evidence, wrote manuscript and edited manuscript. Ya.T. provided guidance for the manuscript and wrote manuscript.

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals

Not applicable.

Informed consent

Not applicable.

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Figures

Figure 1

NAD⁺ biosynthetic pathways. De novo biosynthesis begins with the conversion of tryptophan (Trp) to N-L-formylkynurenine either by indolamine-2,3-dioxygenase (IDO) or tryptophan-2,3-dioxygenase (TDO). After four reaction steps, N-L-formylkynurenine can subsequently be converted to the unstable 2-amino-3-carboxymuconate-6-semialdehyde acid, which can undergo non-enzymatic cyclization to quinolinic acid. The last step of de novo biosynthesis consists of the quinolinate-catalyzed phosphoribosyltransferase (QPRT) formation of NA mononucleotide (NaMN) using PRPP (5-phosphoribosyl 1-pyrophosphate). The Preiss-Handler pathway is initiated by NA phosphoribosyltransferase (NAPRT) to form NaMN. Further, NaMN is converted into adenine dinucleotide NA (NAAD) by the enzymes NMN adenylyltransferase (NMNAT1-3) together with ATP. Finally, NAAD is converted to NAD⁺ via an amidation reaction catalyzed by the enzyme NAD⁺ synthase (NADSYN). Core Recycling and Salvage Pathways are the shortest ways to synthesize NAD⁺ from NAM and NR (2 steps). NAM is converted by the rate-limiting nicotinamide phosphoribosyltransferase (NAMPT) to form NMN using PRPP as a cosubstrate. NMN is also a product of NR phosphorylation by NR kinases (NRK1-2). The subsequent conversion of NMN to NAD⁺ is catalyzed by the NMNAT1-3 enzymes. Further, the synthesized NAD⁺ is used in the work of such enzymes as sirtuins, PARPs, CD38 / 157. NR, nicotinamide riboside; NAM, nicotinamide; NMN, NAM mononucleotide; AFMID, kynurenine formamidase; KMO, kynurenine 3-monooxygenase; KYNU, tryptophan 2,3-dioxygenase; 3HAO, 3-hydroxyanthranilic acid oxygenase

Figure 2

The balance of NAD⁺ is the balance of synthesis, consumption and recirculation in various subcellular compartments (cytosol, nucleus and mitochondria). After entering the cell, NAD⁺ precursors are metabolized by four main pathways (Fig. 1) to NAD⁺. In the cytosol, nicotinamide (NAM) is converted to nicotinamide mononucleotide (NMN) by the intracellular form of NAM phosphoribosyltransferase (iNAMPT). NMN is then converted to NAD⁺ by NMN transferase 2 (NMNAT2) bound to the outer Golgi membrane in the cytoplasm. NAD⁺ is converted to NADH / NADPH during the redox cycle, is also used during aerobic / anaerobic glycolysis and is consumed by NAD-dependent enzymes (SIRT2, PARP1-3, CD38 / 157, SARM1). In mitochondria, NMN is converted to NAD⁺ by NMNAT3. NAD⁺ is used by the TCA cycle to generate ATP and is additionally used by mitochondrial sirtuins 3-5 (SIRT3-5) and PARP1, which generate NAM. Studies show the presence of NAD⁺, NADH and NMN transporters in the mitochondrial membrane, but no specific transporters have yet been identified. It is still not clear whether NAM can be converted back to NMN within the mitochondria, or whether it is transported / diffused from the mitochondria into the cytosol. Inside the nucleus, NMN is converted to NAD⁺ by NMNAT1, while NAD⁺ is consumed here mainly by SIRT1,6,7, PARP1-3, SARM1. As in the cytosol, NAM is returned back to the NMN by iNAMPT

Figure 3

Hypothesis of the impact of NR in the development of doxorubicin cardiomyopathy. Today, it is a popular assumption that the development of doxorubicin (DOX) cardiotoxicity occurs due to the massive generation of ROS, which in turn is caused by a secondary mechanism due to the suppression of topoisomerase 2β. In consequence DNA damage grows, enzymes of its repair, PARPs, CD38 are activated, while the activity of sirtuins and NAMPT (the main pathway of NAD⁺ synthesis through NAM under normal physiological conditions, Core Recycling Pathway) is suppressed, the work of Complex I in the

Electron transport chain is disrupted, using NADH as an electron donor, creating NAD⁺. Damage to Complex 1 and ATP production leads to accumulation of ROS that increase oxidative stress. Separately doxorubicin reduces tight junction formation by decreasing the expression of the occludin (ZO)-1, which can increase doxorubicin levels in the capillary endothelium of the heart muscle. It also reduces the nitric oxide (NO) level by enzymatic inhibition, by reducing the level of endothelin (ET)-1, accumulation of ROS [93]. All of these mechanisms lead to a significant decrease in NAD⁺ levels, the inability of the antioxidant system of the cardiomyocyte to cope with increased oxidative stress, mitochondrial dysfunction, which ultimately leads to apoptosis of endothelial and cardiac cells. The administration of nicotinamide riboside (NR) can significantly increase the level of NAD⁺ through a less energy-intensive synthesis through NRK1 / 2. An increase in the level of NAD⁺ activates the work of sirtuins, which are able to induce antioxidant defense systems that restore mitochondrial biogenesis damaged by oxidative stress and promote the activation of autophagy, which is disturbed by doxorubicin, leading to the accumulation of non-degradable autolysosomes. NMN synthesis via NR inhibits endothelial inflammation and improves NO-dependent function. Activation of endothelial SIRT1 controls endothelial homeostasis and vascular functionality by modulating the activity of endothelial nitric oxide synthase (eNOS), p53, angiotensin II receptor (Ang II), FOXO1 and other mechanisms [94]