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ORIGINAL ARTICLE

Improvement of tissue-specific distribution and biotransformation potential of nicotinamide mononucleotide in combination with ginsenosides or resveratrol

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Abstract

Decreased Nicotinamide adenine dinucleotide (NAD^+) level has received increasing attention in recent years since it plays a critical role in many diseases and aging. Although some research has proved that supplementing nicotinamide mononucleotide (NMN) could improve the level of NAD^+, it is still uncertain whether the NAD^+ level in specific tissues could be improved in combination with other nutrients. So far, a variety of nutritional supplements have flooded the market, which contains the compositions of NMN coupled with natural products. However, the synergy and transformation process of NMN has not been fully elucidated. In this study, oral administration of NMN (500 mg/kg) combined with resveratrol (50 mg/kg) or ginsenoside Rh2&Rg3 (50 mg/kg) was used to validate the efficacy of appropriate drug combinations in mice. Compared with NMN alone, NMN combined with resveratrol could increase the levels of NAD^+ in the heart and muscle by about 1.6 times and 1.7 times, respectively, whereas NMN coupled with ginsenoside Rh2&Rg3 could effectively improve the level of NAD^+ in lung tissue for approximately 2.0 times. Our study may provide new treatment ideas for aging or diseases in cardiopulmonary caused by decreased NAD^+ levels.

KEYWORDS
combination therapy, ginsenosides, LC-MRM, NAD^+, NMN, resveratrol

1 | INTRODUCTION

Nicotinamide adenine dinucleotide (NAD^+), as an essential hydrogen acceptor and cofactor, participated in many biological processes including glycolysis, gluconeogenesis, citrate cycle, fatty acid β-oxidation, and the synthesis of cholesterol, fatty acids, and steroids.[1,2] Several studies have shown that the decrease of NAD^+ is a vital factor in aging.[3–6] Although NAD^+ supplement is self-evident

Abbreviations: ANOVA, analysis of variance; CD73, cluster of differentiation 73; ESI, electrospray ionization; LOD, limit of detection; LOQ, limit of quantification; MRM, multiple reaction monitoring; *m/z*, mass to charge ratio; NAD^+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NMNATs, nicotinamide mononucleotide adenylyltransferases; NMNAT2, nicotinamide mononucleotide adenylyltransferase 2; NR, nicotinamide riboside; NRK, nicotinamide riboside kinase; PBS, phosphate buffered saline; QC, quality control; RSD, relative standard deviation; SEM, standard error of the mean; S/N, signal-to-noise ratio; UHPLC-QqQ-MS, ultra-high performance liquid chromatography with triple quadrupole mass spectrometry.

to prevent aging and related diseases, oral administration of NAD^+ is unable to be utilized by cells directly. Nicotinamide mononucleotide (NMN) as a direct precursor in the salvage pathway is converted into NAD^+ under the catalysis of nicotinamide mononucleotide adenylyltransferases (NMNATs).[7–9] Studies demonstrated that oral administration of NMN at a limit dose of 2666 mg/kg did not lead to any mortality or treatment-related adverse signs,[10] and orally administered NMN could be quickly utilized to enhance energy metabolism through transferring NAD^+ in multiple tissues. Those benefits could serve as a possible therapeutic strategy for controlling different pathological states.[8,11,12] It is necessary to increase NAD^+ levels in specific tissues because the reduction of NAD^+ in tissues, especially in cardiopulmonary tissues, often leads to a variety of diseases including heart failure[1,13] and idiopathic pulmonary fibrosis.[14]

NMN can often be combined with some drugs to produce better efficacy.[15,16] Therefore, based on the idea of drug combination, we hope to promote NAD^+ levels in specific tissues by combining NMN with some natural products. As a polyphenol phytoalexin, resveratrol could decrease oxidative stress, attenuate inflammation, and even regulate the NAD^+-dependent deacetylase sirtuins (SIRTs) and has considerable potential for improving human health and preventing chronic diseases.[17,18] In addition, the therapeutic potential on immune regulation, antitumor, and antiaging of ginsenosides has been in the spotlight.[19] And some ginsenosides could modulate the nicotinamide phosphoribosyltransferase (NAMPT) to maintain mitochondrial function, which is the rate-limiting enzyme in the NAD^+ salvage pathway.[20,21] Although these natural products have the potential to increase NAD^+, knowledge about their combined effects to improve the NAD^+ levels in tissue-specific distribution is limited. Moreover, resveratrol and ginsenoside, as natural products with various biological activities, easy absorption, and nearly no toxicity to the human body,[22–29] also have broad prospects for clinical studies on oral administration with NMN in the future.

In order to evaluate whether the combination of resveratrol or ginsenoside could improve NAD^+ levels in different tissues, an ultra-high performance liquid chromatography with triple quadrupole mass spectrometry (UHPLC-QqQ-MS) method was established in this study. After oral administration of NMN with or without resveratrol or ginsenosides Rh2&Rg3, the distribution of NMN and its metabolites of NAD^+ and nicotinamide riboside (NR) were detected in mice tissues. Our study may offer further support to broaden the therapeutic application or nutritional requirements of synergistic NMN preparations.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

NMN and NAD^+ were purchased from Jiuding Chemical Industry Co. Ltd. NR and ginsenosides Rg3&Rh2 (including 35.7% (20R)-ginsenoside Rg3 and 43.6% (20R)-ginsenoside Rh2) were purchased from Daosifu Biotechnology Co. Ltd. Resveratrol was obtained from Baolong Zhongcheng Biotechnology Co. Ltd. Methanol of MS grade was a product from J.T. Baker, whereas ammonium formate (MS grade) and formic acid (MS grade) were obtained by Sigma-Aldrich and Honeywell Fluka, respectively. Ultrapure water was supplied by the Milli-Q water purification system.

2.2 | Animals

Male C57/BL6 mice at 6–8 weeks of age (20–25 g) were purchased from SPF Biotechnology Co., Ltd with the license of SCXK (Beijing) 2019-0010. All animal experiments were approved by the Division of Animal Control and Inspection, Department of Food and Animal Inspection and Control, Instituto para os Assuntos Cívicos e Municipais (IACM), Macau (Audit Form No.: AL003/DICV/SIS/2017). All the animals were kept in individual ventilated cages at a suitable temperature and humidity and were controlled under 12 h light/dark cycles. Mice had access to diet and water ad libitum. The mice were fasted overnight but with access to water before the experiment.

2.3 | Grouping and treatment

NMN (50 mg/ml), resveratrol (5 mg/ml), and Rg3&Rh2 (5 mg/ml) were individually suspended in phosphate buffered saline (PBS) solution for oral study. The mice were divided into four groups, including (1) control, (2) NMN, (3) NMN and resveratrol combination group, and (4) NMN and ginsenosides Rg3&Rh2 combination group. The control group was given PBS twice orally, and the NMN group was given NMN (500 mg/kg) after oral administration of PBS. The combination groups were orally given resveratrol (50 mg/kg) or ginsenosides Rg3&Rh2 (50 mg/kg) followed by NMN (500 mg/kg). The administration dosage of NMN, resveratrol, and ginsenosides employed in this study was decided according to their reported therapeutic dose mentioned in the literature.[8,10,23,27,28,30–32] Three animals per group were sampled at each timepoint. The animals were sacrificed by cervical dislocation at 1, 2, 4, and 6 h postadministration. The tissue samples (brain, heart, kidney, liver, lung, and muscle) were quickly collected and washed with ice-cold PBS, then immediately utilize for extraction.

2.4 | Sample preparation

The tissues were quickly cut into pieces and homogenized by a high-speed homogenizer with PBS on ice, then centrifuged at 3500*g* for 20 min at 4°C. After removing the supernatant, 40 µl ice acetonitrile:methanol:water (2:2:1 v/v) was added to per milligram homogenized sample and mixed by vortex, then incubated on ice for 20 min. After centrifuging at 16000*g* for 20 min at 4°C, the supernatant was transferred to a new tube and evaporated to dryness by speed vacuum. The residue was redissolved in 100 µl Milli-Q water, vortexed for 2 min, and then centrifugated at 16000*g* for 20 min at 4°C. Then, an aliquot of 1 µl supernatant was injected into the UHPLC system for analysis.

2.5 | LC-MRM conditions

The chromatographic separation was conducted on an Agilent 1290 UHPLC system, equipped with a ZORBAX Eclipse AAA column (4.6×150 mm, 1.5 μ). Gradient elution was achieved by the mobile phase, which consisted of water with 5 mM ammonium formate and 0.05% formic acid (A) and methanol (B), and performed with the following schedule: 2%–5% of B at 0–8 min, 5%–15% of B at 8–15 min, 15%–80% of B at 15–18 min, 80%–100% of B at 18–18.1 min, 100% B at 18.1–20 min, 100–2% of B at 20–20.1 min, and 2% B at 20.1–25 min. The column temperature was set at 40°C, and the flow rate was at 0.3 ml/min.

Quantitative analysis was carried out by Agilent 6460 QqQ mass spectrometer. After optimization, the flow rate of 11 L/min for sheath gas and electrospray ionization (ESI) conditions were run at positive ion mode with a capillary voltage of 4000 V, a nebulizer pressure of 40 psi, and 11 L/min and 325°C for drying gas. For getting a better limit of quantification (LOQ), dynamic multiple reaction monitoring (MRM) mode was adopted. LC-MRM data were collected by Agilent Mass Hunter Workstation Data Acquisition (Version B.07.00) and processed by QqQ Quantitative Analysis software (Version B.06.00).

2.6 | Preparation of aqueous-matched and matrix-matched samples

Standards of NMN, NR, and NAD^+ were thoroughly mixed in Milli-Q-water, then the aqueous-matched samples were prepared in a serial twofold dilution with more than seven concentration levels. The matrix was prepared by pooling different tissues from the control group and followed the same protocol as mentioned in the section in sample preparation. Then, the matrix-matched samples were prepared by spiking the aqueous-matched samples into the tubes containing the dried matrix.

2.7 | Extraction recovery

Quality control (QC) samples at low (1.95 μM), medium (15.63 μM), and high (125 μM) concentrations were used to evaluate the extraction recovery by comparing the signal response of the three analytes spiked into the matrix prior to and after extraction. Each measurement was carried out in three replicates.

2.8 | Method validation

To validate the method, linearity, matrix effect, limit of detection (LOD), LOQ, accuracy, repeatability, and precision were evaluated. All the standard curves were calculated for the log (peak area) against the log (concentration). The signal response of analytes in each matrix-matched sample was subtracted from that in the matrix. Matrix effects were evaluated by comparing the slopes of aqueous-matched and matrix-matched standard curves. The LOD was set at a signal-to-noise ratio (S/N) of 3, and the LOQ was determined by considering an S/N of 10. Accuracy and precision were validated by measurements of six replications of QC samples at low, medium, and high concentrations. Interday precision was evaluated on three separate days at 4°C. Repeatability was validated by measurements nine QC samples, prepared in parallel. Accuracy was expressed as the percentage difference between expected and observed concentration per analytical run. Precision and repeatability were provided by the relative standard deviation (RSD). Acceptable accuracy was within ±15%, and the repeatability, intraday precision, and interday precision were ≤15%.

2.9 | Statistical analysis

The experimental results were presented as mean ± standard error of the mean (SEM) and were obtained from three accumulation concentrations of NMN, NR, or NAD^+, each accumulation concentration is the sum of four independent repeated experiments postadministration 1, 2, 4, and 6 h, respectively. All data were performed by GraphPad Prism 8.02. One-way analysis of variance (ANOVA) was used to analyze the significance of the intergroup differences within the same tissue. And the p value of $<.05$ was considered statistically significant followed by Tukey's multiple comparisons test.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the MRM and retention time settings

It was reported that NMN, NAD^+, and NR were very labile under standard ESI and readily undergo in-source fragmentation.[33] Thus, the poor separation may potentially lead to inaccurate annotation. Moreover, it was difficult for the conventional reverse-phase column to retain and separate these compounds because most of the NAD^+ metabolites are highly polar molecules.[34] Thereby, an amino acid analysis column was chosen to obtain complete baseline separation in the current study.

For NMN and its metabolites, $[M+H]^+$ was selected as the precursor, and the fragmentor voltage and collision energy were optimized to achieve the maximum MS intensity for precursor ion and product ion using standard solutions. After a series of conditional explorations, the optimization MRM conditions were finally established. The optimized MRM settings for the NMN, NR, and NAD^+ were depicted in Table 1.

3.2 | Method validation

3.2.1 | Extraction recovery

Extraction recovery was achieved in the following range from 95.01% to 100.29% for NMN, 106.88% to 119.44% for NR, and 91.13% to 106.99% for NAD^+, respectively (Table 2).

TABLE 1 Multiple reaction monitoring settings and retention time for NMN, NR, and NAD^+

Analyte	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Polarity
NMN	5.87	335.1	123.1	140	8	Positive
NR	6.48	255.1	123.1	80	4	Positive
NAD^+	11.34	664.1	136.1	80	52	Positive

TABLE 2 Extraction recovery for NMN, NR, and NAD^+ in mice tissues

	Recovery (SEM)		
Analyte	Low	Medium	High
NMN	100.29% (6.73)	95.59% (5.34)	95.01% (5.79)
NR	119.44% (1.14)	106.88% (2.43)	112.41% (10.07)
NAD^+	106.99% (8.19)	92.47% (1.35)	91.13% (4.90)

3.2.2 | Linearity and matrix effect

Limits of detection and the range of quantitation were studied for the standard samples, which include NMN, NR, and NAD^+. Standard curves were established with a wide dynamic range (over five orders of magnitude). The analytes show good linearity ($r^2 > .9938$). The slopes of aqueous-matched and matrix-matched standard curves are virtually the same indicating no significant matrix effect[35] (Table 3). Finally, an aqueous-matched standard curve was selected for quantification (Table 4).

3.2.3 | LOD and LOQ

The method showed good sensitivity for detecting NMN, NR, and NAD^+, with LOD and LOQ values determined at 0.0038–0.0076 and 0.0076–0.0153 μM, respectively (Table 4).

3.2.4 | Accuracy, repeatability, and precision

Our results have presented good accuracy, as shown in Table 5, and the accuracy of NMN, NR, and NAD^+ was in the range of 87.3%–91.3%, 86.5%–94.6%, and 80.8%–108.2%, respectively. The intra-precision and interday precision (as RSD) of NMN, NR, and NAD^+ were all within the range of 0.70%–2.96% and 0.79%–4.13%, respectively (Table 5). The RSD of repeatability of the three compounds was all <10.46%. All these results demonstrated that the method was accurate, precise, and repeatable for quantification.

3.3 | The combination of NMN and resveratrol or ginsenosides improves the distribution of NMN in mice tissues

Although NMN is sharply metabolized in plasma (within 30 min),[12] it takes a longer time for transformation in tissues. The NMN concentrations in different tissues at 1, 2, 4, and 6 h were determined after orally administrated NMN alone or combined with resveratrol or Rg3&Rh2. For investigating the tissue-specific manner in mice intuitively, the NMN contents at each point-in-times are presented in Figure 1. In NMN alone group, NMN was mainly enriched in the liver ($p < .001$). The NMN level in heart has been promoted for a 1.39-fold change after being combined with resveratrol ($p < .05$). However, compare with NMN alone, adjuvant ginsenoside Rg3&Rh2 could improve its distribution efficiency in brain, heart, kidney, and lung tissues for 4.14-, 2.31-, 4.47-, and 2.39-fold changes, respectively ($p < .001$).

With the selection of natural products, the combination effect on tissue distribution changes may be different. The action target of resveratrol may be more focused on cardiac,[36] whereas ginsenoside may play a role in multiple organs.[37–40] Compared with resveratrol combination group, the ginsenosides Rg3&Rh2 combination group showed more than 2.01-fold changes in improving the tissue distribution of NMN in brain, heart, kidney, liver, and lung tissues ($p < .01, < .001$).

3.4 | Combination administration improves the distribution of NR in mice tissues

Before entering mammalian cells, NMN is dephosphorylated to produce NR under the action of extracellular receptor cluster of differentiation 73 (CD73), then entered into cells by equilibrative nucleoside transporters. Subsequently, the subsequent NR reconverts to NMN in cells by nicotinamide riboside kinase (NRK).[41,42] As a metabolite of NMN, the concentration of NR remained at a low level in brain, lung, and muscle tissues, respectively (Figure 2). It is noteworthy that there was a high NR level in the kidney, which may be related to the high expression of NRK and CD73 in related tissues.[2] Compared with the NMN group, no significant improvement in NR was observed in the resveratrol combination group except for a 1.93-fold improvement in heart tissues ($p < .001$). However, when combined with Rg3&Rh2, the NR levels were significantly increased in brain, kidney, and liver tissues compared with NMN alone or NMN plus resveratrol ($p < .05, .001$). These results were consistent with the above distribution of NMN in each administration group and suggested that the level of NMN is inseparable from the concentration of NR.

3.5 | The combined administration of NMN and resveratrol and ginsenosides improves the distribution of NAD^+ in mice tissues

It was known that the reduction of NAD^+ is associated with several diseases and pathologic states, and the supplementation of NMN directly is safe and feasible for improving NAD^+ in the body.[8]

TABLE 3 Calibration data of UHPLC-QqQ MS and matrix effect for comparison of slopes

	Aqueous-matched standard curve			Matrix-matched standard curve			
	Equation ($y = ax + b$)			Equation ($y = ax + b$)			Matrix effect
Analyte	*a*	*b*	r^2	*a*	*b*	r^2	*a* (%)
NMN	1.09	3.59	0.9982	1.12	3.91	0.9998	98.09
NR	0.83	4.68	0.9979	0.81	4.91	0.9992	101.96
NAD^+	0.96	3.06	0.9938	0.91	3.19	0.9974	105.19

Note: y = log (peak area), a = slope, x = log (concentration), b = intercept and r^2 = correlation coefficient. Matrix effects are expressed as the ratio between the slopes of matrix-matched standard curve and aqueous-matched standard curve.

TABLE 4 Linear equations, correlation coefficients (r^2), linear ranges, limits of detection, and limit of quantification (LOD/LOQ) for NMN, NR, and NAD^+

Analyte	Linear equation	r^2	Linear range (μM)	LOD (μM)	LOQ (μM)
NMN	$y = 1.0938x + 3.5878$	0.9982	0.0076–500	0.0038	0.0076
NR	$y = 0.8284x + 4.6812$	0.9979	0.0076–500	0.0038	0.0076
NAD^+	$y = 0.9551x + 3.0634$	0.9938	0.0153–500	0.0076	0.0153

TABLE 5 Precision, accuracy, and repeatability for NMN, NR, and NAD^+

	NMN			NR			NAD^+		
Parameters	QCL	QCM	QCH	QCL	QCM	QCH	QCL	QCM	QCH
Precision									
Intraday (RSD%)	1.55	0.99	0.88	2.18	1.32	1.77	2.96	2.38	0.70
Interday (RSD%)	2.05	1.02	0.93	2.98	1.18	1.81	4.13	2.49	0.79
Accuracy (%)	91.3	87.3	88.5	86.5	94.6	81.8	108.2	80.8	92.7
Repeatability (RSD%)	8.31	4.01	2.87	9.25	5.73	2.78	4.01	10.21	10.46

As shown in Figure 3, the levels of NAD^+ were significantly increased in the liver ($p < .001$) and kidney ($p < .001$) after oral administration of NMN alone. The result suggested that NMN supplementation could improve the levels of energy metabolism in liver and kidney tissues. Surprisingly, compared with NMN administration alone, the combination of NMN and resveratrol increased the NAD^+ levels in the heart and skeletal muscle by 1.59-fold ($p < .001$) and 1.72-fold ($p < .001$), respectively. The benefit of NAD^+'s supplementation in the heart was to block the symptoms of agonist-induced cardiac hypertrophy and protect the myocardial ischemia–reperfusion injury.[43–45] In addition to heart, NAD^+ abundance positively correlated with muscle functioning.[46] NAD^+ repletion could protect mdx muscle from damage, inflammation, and fibrosis.[47] On the other hand, the NAD^+ level in lung tissue was significantly increased by 1.97 times when NMN was combined with Rg3&Rh2 ($p < .001$), and this benefit may be used for counteracting the reduction in autophagy in cigarette smoke-induced senescence of alveolar epithelial cells.[48]

In our results, the NAD^+ level in different tissues increased after being coupled with resveratrol or ginsenosides Rg3&Rh2. P53, as a key role in the aging process, controls cell growth as well as apoptosis.[49] As an NAD^+ synthetase, nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) has been confirmed to be a downstream target gene of p53.[50] Resveratrol has been reported to induce p53 activation.[51] Moreover, it was reported that ginsenosides act on the adjacent regions of the p53 DNA-binding pocket, improve the stable binding of p53 with DNA, and regulate the expression of downstream proteins.[52] Therefore, we speculated that oral administration of Rg3&Rh2 or resveratrol may improve the function of p53 and indirectly increase the biotransformation level of NAD^+ by enhancing the expression of NMNAT2.

This study was the first to investigate the metabolism and distribution of NMN, NR, and NAD^+ in multiple tissues after oral administration of NMN combined with resveratrol or ginsenoside. However, there are also a few limitations to our study. For instance, small sample sizes may limit the reliability of statistical evaluation. Moreover, only adult mice were employed in this study to determine the tissue-specific distribution and biotransformation of NMN. Therefore, in our further study, it is worthy to extrapolate the study to different age groups, especially for the aged population, as well as the model groups with cardiopulmonary diseases. Furthermore, larger sample size may be necessary for further confirmation for our findings.

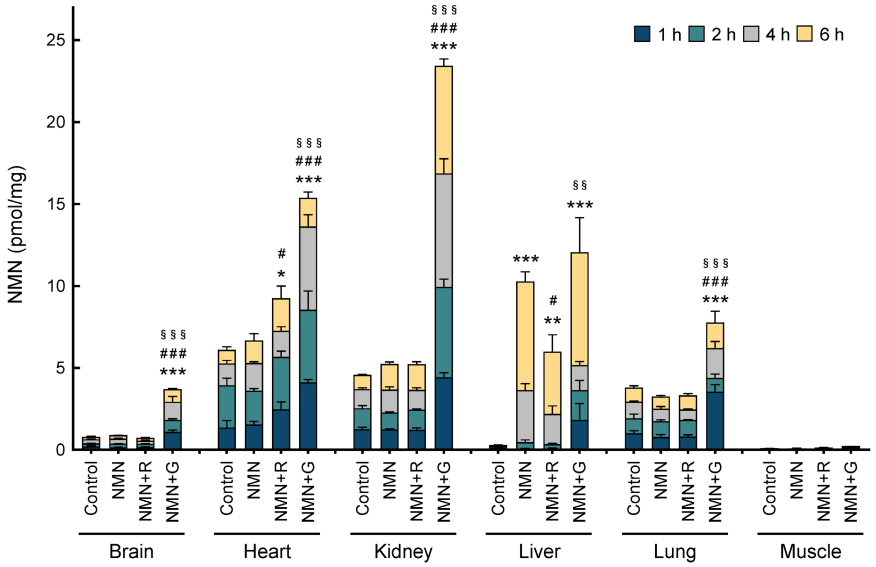

FIGURE 1 The bar chart showing the distribution of nicotinamide mononucleotide (NMN) in different mice tissues ($n = 3$) among control as well as treatment groups upon oral administration of NMN, NMN + R, and NMN + G for 1, 2, 4, and 6 h, respectively. Data for each time point were shown as mean ± standard error of the mean (SEM). Control group, oral administration of PBS; NMN group, oral administration of NMN (500 mg/kg) alone; NMN + R group, oral administration of resveratrol (50 mg/kg) followed by NMN (500 mg/kg); NMN + G group, oral administration of ginsenosides Rg3&Rh2 (50 mg/kg) followed by NMN (500 mg/kg). Comparisons between each group among each tissue were performed according to the accumulation of NMN within 6 h. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ when comparing NMN, NMN + R, and NMN + G with the control group, respectively; $^{\#}p < .05$, $^{\#\#\#}p < .001$ when comparing NMN + R and NMN + G with the NMN group, respectively; $^{\S\S}p < .01$, $^{\S\S\S}p < .001$ when comparing NMN + R group with NMN + G group. The distribution of NMN in mice tissues for combination administration within 6 h.

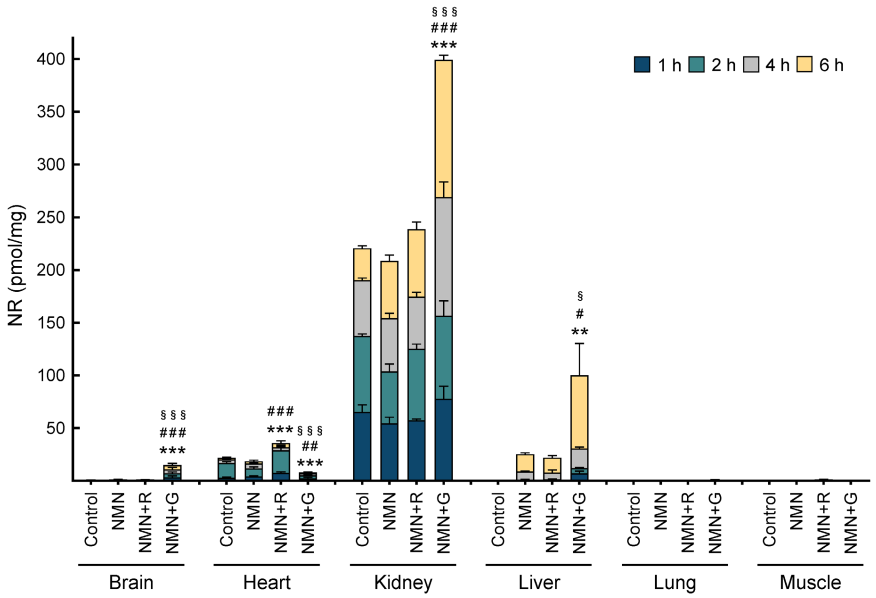

FIGURE 2 The bar chart showing the distribution of nicotinamide riboside (NR) in different mice tissues ($n = 3$) among control as well as treatment groups upon oral administration of NMN, NMN + R, and NMN + G for 1, 2, 4, and 6 h, respectively. Data for each time point were shown as mean ± standard error of the mean (SEM). Control group, oral administration of PBS; NMN group, oral administration of NMN (500 mg/kg) alone; NMN + R group, oral administration of resveratrol (50 mg/kg) followed by NMN (500 mg/kg); NMN + G group, oral administration of ginsenosides Rg3&Rh2 (50 mg/kg) followed by NMN (500 mg/kg). Comparisons between each group among each tissue were performed according to the accumulation of NR within 6 h. $^{**}p < .01$, $^{***}p < .001$ when comparing NMN, NMN + R, and NMN + G with the control group, respectively; $^{\#}p < .05$, $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ when comparing NMN + R and NMN + G with the NMN group, respectively; $^{\S}p < .05$, $^{\S\S\S}p < .001$ when comparing NMN + R group with NMN + G group. The distribution of NR in mice tissues for combination administration within 6 h.

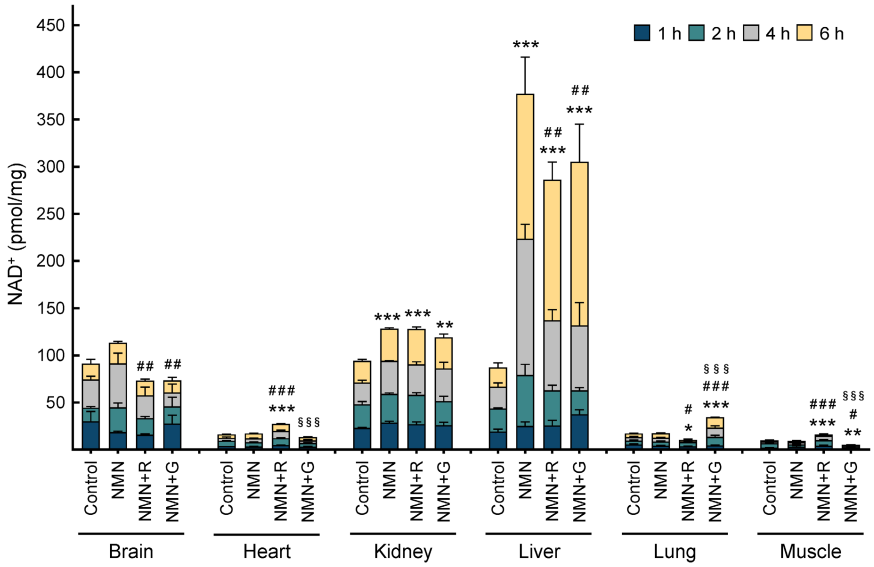

FIGURE 3 The bar chart showing the distribution of nicotinamide adenine dinucleotide (NAD^+) in different mice tissues ($n = 3$) among control as well as treatment groups upon oral administration of NMN, NMN + R, and NMN + G for 1, 2, 4, and 6 h, respectively. Data for each time point were shown as mean ± standard error of the mean (SEM). Control group, oral administration of PBS; NMN group, oral administration of NMN (500 mg/kg) alone; NMN + R group, oral administration of resveratrol (50 mg/kg) followed by NMN (500 mg/kg); NMN + G group, oral administration of ginsenosides Rg3&Rh2 (50 mg/kg) followed by NMN (500 mg/kg). Comparisons between each group among each tissue were performed according to the accumulation of NAD^+ within 6 h. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$ when comparing NMN, NMN + R, and NMN + G with the control group, respectively; $^{\#}p < .05$, $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ when comparing NMN + R and NMN + G with the NMN group, respectively; $^{\S\S\S}p < .001$ when comparing NMN + R group with NMN + G group. The distribution of NAD^+ in mice tissues for combination administration within 6 h.

4 | CONCLUSION

In this study, a UHPLC-MRM method was established to quantify the levels of NMN, NR, and NAD^+ in C57/BL6 mice tissues. Our results showed that combination with resveratrol could effectively increase the levels of NAD^+ in the heart and skeletal muscle compared with NMN alone. Dietary NMN combined with ginsenoside Rh2&Rg3 could improve the distribution of NMN in brain, heart, kidney, and lung tissues and increase the NAD^+ level in lung tissue more effectively than ingestion of NMN alone. Thus, our study elucidates an insight into combination therapy for supplementing NAD^+ in daily or NAD^+ metabolic disorder levels, especially in cardiopulmonary failure diseases.

AUTHORS CONTRIBUTION

Methodology, L.-B.B. and L.-F.Y.; validation, L.-B.B. and L.-F.Y.; formal analysis, L.-B.B.; investigation, L.-B.B., L.-F.Y., T.-T.T., and W.-H.C.; data curation, L.-B.B.; writing—original draft preparation, L.-B.B.; writing—review and editing, L.-F.Y., W.Z., and Z.-H.J.; visualization, L.-B.B.; supervision, L.-F.Y. and Z.-H.J.; project administration, L.-B.B.; funding acquisition, Z.-H.J. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All animal experiments were approved by the Division of Animal Control and Inspection, Department of Food and Animal Inspection and Control, Instituto para os Assuntos Cívicos e Municipais (IACM), Macau (Audit Form No.: AL003/DICV/SIS/2017).

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

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