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Research Paper

N-acetyl-L-cysteine ameliorates the $PM_{2.5}$-induced oxidative stress by regulating SIRT-1 in rats

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ABSTRACT

Silent information regulator 1 (SIRT1), a class III histone deacetylase, plays a major role in combating cellular oxidative stress injury. However, the role of SIRT1 in oxidative stress induced by particulate matter remains unclear. A total of 32 healthy male Sprague–Dawley rats were divided into $PM_{2.5}$, $PM_{2.5}$ + NAC, filtered air (control), and filtered air + NAC (NAC control) groups. The expressions of MnSOD, SIRT1, and FOXO3a were examined at both transcriptional and protein levels. The expression levels of MnSOD, SIRT1, and FOXO3a reduced significantly ($P < 0.05$) in the $PM_{2.5}$ group as compared to the control group. However, their expression levels were increased after NAC intervention. These results suggested that SIRT1 exerted a protective effect against $PM_{2.5}$-induced respiratory oxidative damage by regulating the expression of FOXO3a. NAC can activate SIRT1 and exert an anti-oxidative role in $PM_{2.5}$-induced oxidative injury.

1. Introduction

Particulate matter, especially ≤2.5 μm aerodynamic diameter ($PM_{2.5}$), is the primary air pollutant that is hazardous to human health (Xing et al., 2016; Zhao et al., 2013). Oxidative stress represents a series of physiological and pathological processes due to the imbalance between the oxidation and anti-oxidation system (Di Meo et al., 2016). Several studies suggested that the oxidative stress is one of the major pathogenic pathways of $PM_{2.5}$ (Kelly and Fussell, 2015; Moller et al., 2014). After $PM_{2.5}$ enters into the respiratory system, it induces an abundant production of intracellular reactive oxygen species (ROS) (Brunet et al. 2004), reduces the content of the antioxidative enzymes, decreases the enzymatic activity, and activates the inflammatory cells that generate excessive ROS and reactive nitrogen species (RNS), ultimately disrupting the internal environment and leading to cell aging and death (Vattanasit et al., 2014).

Sirtuins (SIRT), class III histone deacetylases (HDACs), seem to have evolved to respond to a variety of stresses, thereby emerging as key anti-aging molecules and regulators in several chronic diseases (Yao and Rahman, 2012). SIRT1, homologous to yeast silent information regulator 2, is the most extensively studied sirtuin as the key molecule in a prolonged lifespan in response to caloric restriction and resistance to oxidative stress (Chang and Guarente, 2014; Michan and Sinclair, 2007). The SIRT1-mediated deacetylation of molecules such as FOXO3, p53, and NF exhibits a profound effect on mitochondrial function, apoptosis, and inflammation (Chang and Guarente, 2014; Lin and Fang, 2013). These physiological processes and functions are critical in the determination of lifespan and outcome following injury (Poulose and Raju, 2015). Forkhead box class O 3a (FOXO3a) is a member of the FOXO subfamily, identified as transcription factors with forkhead DNA binding domains that regulate the expression of several genes involved in diverse processes, such as apoptosis, cell cycle progression, vascular remodeling, development, senescence, oxidative stress resistance, innate immune homeostasis, and inflammation (Watroba et al., 2012). Brunet et al. 2004 found that SIRT1 deacetylates and regulates the expression of FOXO3a in response to oxidative stress as demonstrated by the oxidative damage in the rat heart by myocardial ischemia (Brunet et al., 2004). However, whether $PM_{2.5}$-induced oxidative stress can alter the expression of SIRT1 in rat lung tissue and if the resulting changes in the expression of SIRT1 can play an anti-oxidative role by regulating the expression of FOXO3a is yet to be elucidated. N-acetylcysteine (NAC), a well-known antioxidant, has been shown to minimize the oxidative stress and its downstream effects (Kerksick and Willoughby, 2005). Whether NAC can protect the lung tissue from the $PM_{2.5}$-induced oxidative damage and if this protective function is related to SIRT1 is poorly understood. Thus, the present study investigated the altered expression of SIRT1 in the lung tissue of rats in the process of $PM_{2.5}$-inducced oxidative stress and explored the

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interventional effect of NAC.

2. Materials and methods

2.1. Materials

Acetylcysteine effervescent tablets were purchased from CONBA Bio-Pharm Co., Ltd (Zhejiang, China). The antibodies against SIRT1, FOXO3a, and MnSOD were obtained from Abcam (Cambridge, UK). RT-PCR Kit was purchased from TaKaRa (Japan). The MDA detection TBA Kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals

A total of 32 healthy male Sprague–Dawley (SD) rats provided by the Laboratory Animal Center of Hebei Medical University were divided into $PM_{2.5}$, $PM_{2.5}$ + NAC, filtered air (control), and filtered air + NAC (control + NAC) groups. The $PM_{2.5}$ group was placed in a 10 m^2 room at the scientific research building in the Second Hospital of Hebei Medical University. The room had a large window (about 200 × 250 cm^2) with a hole that was kept open for ventilation. The $PM_{2.5}$ + NAC group was placed in the same room, and the rats were gavaged 150 mg/kg/day NAC. The control group rats were placed in a sealed room that was equipped with an air purifier turned "on". The control + NAC group rats inhaled the filtered clean air similar to the control group and were gavaged 150 mg/kg/day NAC simultaneously. For each group, $PM_{2.5}$ and control rats were exposed to either $PM_{2.5}$ or filtered air for 24 h/day for 12 consecutive weeks and gavaged with NAC solvents. All rats were maintained at a uniform temperature of 18 ± 1 °C and humidity. The $PM_{2.5}$ detector monitored and recorded the $PM_{2.5}$ concentration in the indoor and outdoor air, as well as, the air purifier room every 4 h daily, and the average $PM_{2.5}$ concentration was calculated. The rats were euthanized by femoral vein bloodletting after anesthetization using pentobarbital sodium on the last day of exposure. All experiments for animal studies were performed according to the standards established by the United States Animal Welfare Act, set forth by the National Institutes of Health guidelines. All the procedures involving animals were approved by the Animal Care and Use Committee of the Hebei Medical University.

2.3. Histopathology and morphometry

Lung tissue samples were fixed overnight in 4% paraformaldehyde at 4 °C and routinely processed for paraffin embedding. Then, the histological sections (5 μm) of the paraffin-embedded tissues were prepared and stained with hematoxylin-eosin (H&E). Images (×200 magnification) were acquired by microscopy (Olympus, Japan). For assessing the severity and extent of inflammation on the lung tissue, a semi-quantitative pathological scoring method was employed (Cherniack et al., 1991; Hyde et al., 1992). Grade I: normal lung tissue, obvious inflammatory cells infiltration, fibrous tissue hyperplasia cannot be visualized, and alveolar structures are normal; Grade II: small mass of inflammatory cells can be seen in the lung field, pulmonary alveoli show a few abnormal changes, and no fibrous tissue hyperplasia was observed; Grade III: a large area of inflammation can be found in the lung field, distinct infiltration by inflammatory cells, and a small part of fibrous tissue hyperplasia. Some normal lung tissue can yet be discovered; Grade IV: severe inflammatory changes and clusters of inflammatory cells are visible. Pulmonary alveoli show morphological changes, infiltration of inflammatory cells, and necrosis of lung tissue expansion.

2.4. MDA content analysis

Malondialdehyde (MDA), an indicator of lipid peroxidation, was determined in the rat serum using the MDA kit according to the manufacturer's instructions. The MDA levels were expressed as μmol/g protein.

2.5. Real time polymerase chain reactions (RT-PCR)

Total RNA was isolated from the lung tissues using RNAiso Plus Kit (TaKaRa Bio, Japan). The cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's instructions. RT-PCR conditions were as follows: 95 °C for 30 s, (95 °C, 5 s, 60 °C, 34 s) × 40 cycles, with a final analysis of the melting curve at 60–95 °C. The primer pairs were as follows (forward and reverse, respectively):

MnSOD, 5′-TAAGGGTGGTGGAGAACCCA-3′ and 5′- CTGTAAGCGA CCTTGCTCCT −3′;

SIRT1, 5′-AGGGAACCTCTGCCTCATCT-3′ and 5′- AGGGAACCTCT GCCTCATCT −3′;

FOXO3a, 5′-TGAGGAAAGGGGAAATGGGC-3′ and 5′- CGTGGGAGT CACAAAGGTGT −3 ';

GAPDH, 5′-ACTCTGTGTGGATTGGTGGC-3′ and 5′- AGAAAGGGTG TAAAACGCAGC −3′.

The expression level of each gene was normalized to that of *GAPDH* and expressed as a mean of those acquired from the healthy controls by the comparative $2^{-\Delta\Delta Ct}$ method.

2.6. Western blot analysis

Isolated lung tissue cubes were lysed by RIPA buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, and 0.5% sodium deoxycholate. The lysate was homogenized, and the supernatant was obtained by centrifugation. The protein concentration was measured by the Bradford assay. An equivalent amount of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. Then, the membranes were blocked with 5% non-fat milk in PBS with 0.05% Tween-20 (PBST) for 1 h at room temperature, washed briefly, and probed with monoclonal antibodies against MnSOD, SIRT1, FOXO3a (1:300 in 5% BSA in PBST), and GAPDH (1:1000) overnight at 4 °C followed by incubation with a horseradish peroxidase-conjugated goat anti-rat antibody (1:3000). The immunoreactivity bands were detected using enhanced chemiluminescence (ECL, Beyotime Biotechnology, China) according to the manufacturer's instructions. The Image J densitometry software (Version 1.41; National Institutes of Health, Bethesda, MD, USA) was used for the quantitative densitometric analysis of the signals.

2.7. Statistical analysis

Statistical analysis was performed using the SPSS software version 22.0 (IBM, Chicago, USA). The daily average particulate concentration parameters were described as median (inter-quartile range), and the statistical significance of differences between the groups was assessed by the Kruskal–Wallis test. Other data were reported as the mean value ± SD obtained from at least three independent experiments. ANOVA was performed to evaluate the difference between the means, followed by LSD posthoc test. P-value < 0.05 was considered statistically significant.

3. Results

3.1. Evaluation of air quality for $PM_{2.5}$ exposure

During 2015.2.1–2015.4.30, the daily $PM_{2.5}$ average concentration of the $PM_{2.5}$ exposure was estimated as 64.75 μg/m^3 indoors (equivalent to the concentration that the $PM_{2.5}$ group actually inhaled) and 67.4 μg/m^3 outdoors. No statistically significant difference was

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