Cardiovascular Toxicology (2019) 19:178–190
https://doi.org/10.1007/s12012-018-9488-5

Effects of Ambient Atmospheric $PM_{2.5}$, 1-Nitropyrene and 9-Nitroanthracene on DNA Damage and Oxidative Stress in Hearts of Rats

Lifang Zhao[1] · Li Zhang[1] · Minghui Chen[1] · Chuan Dong[1] · Ruijin Li[1] · Zongwei Cai[2]

Published online: 12 November 2018

Abstract
Exposure to fine particulate matter ($PM_{2.5}$) increased the risks of cardiovascular diseases. $PM_{2.5}$-bound 1-nitropyrene (1-NP) and 9-nitroanthracene (9-NA) are released from the incomplete combustion of fossil fuels and derived from polycyclic aromatic hydrocarbons (PAHs). The toxicities of 1-NP and 9-NA are mainly reflected in their carcinogenicity and mutagenicity. However, studies of $PM_{2.5}$-bound 1-NP and 9-NA on the cardiac genotoxicity are limited so far. In this study, histopathology, DNA damage, DNA repair-related gene expression, and oxidative stress were investigated in the hearts of male Wistar rats exposed to $PM_{2.5}$ [1.5 mg/kg body weight (b.w.)] or three different dosages of 1-NP (1.0×10^{-5}, 4.0×10^{-5}, and 1.6×10^{-4} mg/kg b.w.) or 9-NA (1.3×10^{-5}, 4.0×10^{-5}, and 1.2×10^{-4} mg/kg b.w.). The results revealed that (1) $PM_{2.5}$, higher dosages of 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w.) and 9-NA (4.0×10^{-5} and 1.2×10^{-4} mg/kg b.w.) caused obvious pathological responses and DNA damage (DNA strand breaks, 8-OHdG formation and DNA–protein cross-link), accompanied by increasing OGG1 and GADD153 expression while inhibiting MTH1 and XRCC1 expression in rat hearts. Also, they elevated the hemeoxygenase-1 (HO-1), glutathione *S*-transferase (GST), and malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity compared with the control. (2) The lowest dosages 1-NP or 9-NA could not cause DNA damage and oxidative stress. (3) At the approximately equivalent dose level, $PM_{2.5}$-induced DNA damage effects were more obvious than 1-NP or 9-NA along with positive correlation. Taken together, heart DNA damage caused by $PM_{2.5}$, 1-NP and 9-NA may be mediated partially through influencing the DNA repair capacity and causing oxidative stress, and such negative effects might be related to the genotoxicity $PM_{2.5}$, 1-NP, and 9-NA.

Keywords Fine particulate matter · 1-Nitropyrene · 9-Nitroanthracene · DNA damage · Oxidative stress · Rat heart

Introduction

Outdoor atmospheric particulate matter is an environmental risk factor and has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [1]. Fine particulate matter ($PM_{2.5}$) is a typical air pollutant, derived from a wide range of coal combustions, diesel exhausts (DEs), biomass burning, etc. The inhalation of $PM_{2.5}$ makes it easier to transfer from lung to the circulatory system, and to cause severe cardiopulmonary diseases [2]. Epidemiological studies have revealed that exposure to $PM_{2.5}$ is associated with an increased risk of cardiovascular diseases [3, 4], such as ischemic heart disease, heart rhythm disturbances, and heart failure.

The harm of $PM_{2.5}$ to human health mainly depends on its exposure concentration and compositions. $PM_{2.5}$ contains complex components with carbon black, water-soluble

Handling Editor: Travis Knuckles.

✉ Ruijin Li
lirj@sxu.edu.cn

✉ Zongwei Cai
zwcai@hkbu.edu.hk

[1] Institute of Environmental Science, Shanxi University, 92 Wucheng Road, Taiyuan 030006, Shanxi Province, People's Republic of China

[2] State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Jiulong, Hong Kong SAR, China

anions, metals, and organic matters, etc. Among components of urban air $PM_{2.5}$, nitro-polycyclic aromatic hydrocarbons (NPAHs) are a class of widespread genotoxic environmental contaminants [5], formed by the incomplete combustion in the coal, gasoline and biomass burning, or through photochemistry reaction with PAHs and nitrogen oxide in atmosphere. It had been proven that many NPAHs are carcinogenic and/or mutagenic [6, 7]. In particularly, as the derivatives of PAHs, NPAHs exhibit higher direct-acting mutagenicity than that of PAHs [8]. Recently, high concentrations of NPAHs in $PM_{2.5}$ were observed in some cities in China, Russia, Korea, and Japan [9]. A research from Jinan of China reported that the incremental lifetime cancer risk and total risk of NPAHs on hazy days and the dust storm episodes were higher than those on clear days [10]. Considering the potential adverse health effects of $PM_{2.5}$ and NPAHs, the studies of genotoxic effects of $PM_{2.5}$-bound NPAHs are very necessary and important.

Among NPAHs, higher levels of 1-nitropyrene (1-NP) and 9-nitroanthracene (9-NA) have been examined in total $PM_{2.5}$-bound NPAHs [11]. 1-NP has been defined by IARC as Group 2A in view of its probably carcinogenicity to humans [12]. Toxicology research in vitro pointed out that 1-NP may mediate the genotoxic and cytotoxic effects through decreasing cell viability and increasing DNA damage, endoplasmic reticulum stress and intracellular reactive oxygen species (ROS) levels [13]. It was reported that DE, a significant contributor to the toxicity associated with $PM_{2.5}$, could result in cardiovascular dysfunction [14]. Interestingly, the metabolites of 1-NP were detected in urinary samples from the taxi drivers for exposure to DE and had been proposed as biomarkers for exposure to DE [15], suggesting potential associations between $PM_{2.5}$-bound 1-NP and adverse cardiovascular effects. 9-NA is predominately produced in coal combustion and biomass burning process, and its concentration was the highest in all the $PM_{2.5}$-bound NPAHs in Taiyuan, China [16]. Despite that the IARC classified 9-NA as Group 3, namely that 9-NA has uncertain carcinogenicity to humans, 9-NA showed a weak mutagenic activity in *Salmonella typhimurium* strain mutagenic test [17].

There are growing evidences about toxicological mechanisms of $PM_{2.5}$ up to now [18]. Most of these studies focused on $PM_{2.5}$-induced oxidative stress, inflammation, and genotoxicity mainly using lung tissues or airway epithelial cells as experimental materials. Although some experimental studies uncovered that the mitochondrial damage, oxidative stress, inflammation, and apoptosis were potentially important mechanisms for the $PM_{2.5}$-induced heart injury [19, 20], the detailed toxicological studies of $PM_{2.5}$ in the heart were limited currently. With the rapid development of studies about the concentration, source apportionment and exposure risk assessment of $PM_{2.5}$-bound NPAHs in atmosphere [8–10, 16, 21], $PM_{2.5}$-bound NPAHs have aroused people's great concern [22] and the toxicological data on NPAHs are urgently needed for accurate risk assessments. As for 1-NP, some studies revealed that it caused oxidative stress, abnormal metabolic enzyme expression, inflammation, DNA damage, intracellular Ca^{2+}-level elevation and apoptosis in rat lung tissue, human bronchial epithelial cells, human vascular endothelial cells or mouse liver cells via activating specific signaling pathways [13, 23–27]. Relatively, the toxicological data of 9-NA are less. Several studies had shown that 9-NA-induced weak mutagenic activity in strains TA98 and TA100 [17], while 9-NA caused oxidative stress and DNA damage in rat lungs [28], suggesting that 9-NA should make certain toxicity contributions to such adverse effects of $PM_{2.5}$. Taken together, the understanding of the underlying mechanisms by which $PM_{2.5}$ and its component NPAHs induced the heart injury is insufficient and more detailed studies are highly needed.

The excess ROS may attack DNA molecule and induced DNA breaks and oxidative damage, and form DNA oxidation products like 8-hydroxy-2′-deoxyguanosine (8-OHdG). When oxidation level exceeds the antioxidative defenses and the damaged DNA is not promptly repaired, the DNA injury and biological/or pathological consequences tend to be easily caused [29–32]. For instance, a significant increase in DNA damage and oxidative stress was observed in peripheral blood leukocytes of coronary artery disease patients compared to the levels in healthy controls [30]. Oxidative stress-induced DNA damage has been demonstrated to be a critical mechanism of action of urban $PM_{2.5}$ pollution [33]. As an important coal-based heavy industry city in Shanxi Province of China, Taiyuan has been suffering the serious problem of ambient $PM_{2.5}$ pollution along with $PM_{2.5}$-bound NPAHs pollution, especially during the winter [16, 34]. In this study, we determined that the effects of $PM_{2.5}$ and NPAHs representatives (1-NP and 9-NA) on rat heart DNA damage were caused by DNA damage susceptibility gene activation and DNA repair gene inhibition, and the enhanced pathologic responses and oxidative stress were mediated by antioxidant enzymes.

Materials and Methods

$PM_{2.5}$ Sample Preparation

$PM_{2.5}$ sampling site locates on the campus of Shanxi University in Taiyuan, China. $PM_{2.5}$ concentrations were measured using a DustTrakTM II Aerosol Monitor (TSI Inc., USA), and daily $PM_{2.5}$ samples were collected on quartz fiber filters (QFFs) using a $PM_{2.5}$ high-volume air sampler (Thermo Anderson, USA), with a pump flow rate of 1.13 m^3/min. During sampling, $PM_{2.5}$ daily

mass concentrations ranged from 39 to 121 μg/m^3. Some NPAHs were detected and the mass concentrations of Σ-nitropyrene, Σ-nitrofluorene, Σ-nitrochrysene, and Σ-nitroanthracene on $PM_{2.5}$ samples were shown from 0.38 to 3.04 ng/m^3, 0.21 to 0.43 ng/m^3, 0.19 to 2.38 ng/m^3, and 9.55 to 16.52 ng/m^3, respectively.

$PM_{2.5}$ filters were cut and surged in Milli-Q water with sonication, and the extracting solutions were dried under freeze vacuum and made into powder. $PM_{2.5}$ suspensions in physiological saline were prepared under sonication prior to animal experiment. 1-NP or 9-NA, purchased from AccuStandard Inc., USA, was dissolved in 5% dimethylsulfoxide (DMSO), respectively.

Animal and Treatment Protocols

Male Wistar rats from the Animal Center of Hebei Medical University (Shijiazhuang, China) were randomly divided into eight parallel groups ($n = 5$ each group): (1) DMSO control (5% DMSO), (2) $PM_{2.5}$ treatment group (1.5 mg/kg b.w. $PM_{2.5}$), (3)–(5) 1-NP groups (1.0×10^{-5}, 4.0×10^{-5}, 1.6×10^{-4} mg/kg b.w. 1-NP in 5% DMSO), (6)–(8) 9-NA groups (1.3×10^{-5}, 4.0×10^{-5}, 1.2×10^{-4} mg/kg b.w. 9-NA in 5% DMSO). The animals were received 5% DMSO, $PM_{2.5}$ suspensions, 1-NP solutions or 9-NA solutions with the same volume (0.5 mL) by intratracheal instillation, which were performed respectively for 1/2 days for consecutive 10 days. All animal handling procedures followed the Ethics Committee of Scientific Research in Shanxi University, China.

The treatment dosage selection and calculation methods of $PM_{2.5}$, 1-NP and 9-NA were based on our previous studies [24, 28], in which 1.5 mg/kg b.w. of $PM_{2.5}$ in rat was equivalent to air $PM_{2.5}$ exposure levels of the orange alert criterion (500 μg/m^3) of haze in China. Meanwhile, according to the approximate proportion of mass concentration of NPAHs in wintertime $PM_{2.5}$ (ranging 9×10^{-6} to 1×10^{-4}) [11], three 1-NP or three 9-NA dosages above were selected and used to be comparable to dosage of 1.5 mg/kg b.w. $PM_{2.5}$ in this study to evaluate 1-NP and 9-NA toxicity.

After the last treatment, the animals in eight groups were euthanized and sacrificed. Heart tissues were excised into small pieces. A piece of tissue/per rat was immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH = 7.4) for the haematoxylin and eosin (HE) staining. Then a piece of fresh heart tissue per rat was minced and ground to obtain the single cell suspension for the comet assay, another partial heart tissue was cut and homogenized for ELISA and biochemical analysis, and the rest was quickly frozen in liquid nitrogen and then stored at − 80 °C for mRNA and protein measurement.

Comet Assay and DPC Measurement

Heart single cell suspensions were prepared for every rat sample and the alkaline comet assay was performed following our previous study method [28]. In brief, the slides containing "sandwich gel" (first layer: 1% normal melting-point agarose, second layer: 0.65% low melting-point agarose + cells, third layer: 0.65% low melting-point agarose) were prepared. After electrophoresis and staining with 100 μL 4S Red Plus (Sangon, Shanghai, China 1:10,000 dilution), the slides were examined and the DNA damage markers such as the percentage of tail DNA (% Tail DNA), tail length, and olive tail moment (OTM) in the hearts of different group were calculated by a Comet Assay Software Project (CASP, CASP, version 1.2.3 beta1).

The rat heart samples were homogenized in ice-cold physiological saline and then centrifuged for 10 min at 3000 rpm (4 °C) to obtain the tissue supernatants. The levels of DNA–protein crosslinks (DPCs) in heart supernatants were detected by the sodium dodecylsulfate (SDS)–KCl fluorescence spectrometry as described previously [35]. The heart supernatant was treated with SDS–KCl system and then centrifuged to separate the free DNA and the SDS–KCl precipitate contains the protein and DPC complexes. After staining with Hoechst 33258, fluorescence signals of free DNA and DPC samples were measured (Ex: 350 nm, Em: 460 nm) by using a fluorescence spectrophotometer (Hitachi F-4500, Tokyo, Japan). The ratio of the fluorescence of DPC to that of the total DNA (free DNA plus DPC) was calculated, and the results were expressed as percentage of DPC on total DNA.

ELISA Assay

After homogenate and centrifugation, the heart tissues supernatants in different groups were used to measure the levels of 8-oxoguanine-DNA glycosylase (OGG1), heme oxygenase 1 (HO-1) and 8-OHdG with special ELISA kits (R&D Company Ltd., USA), according to the manufacturer's instructions. The ELISA of 8-OHdG utilizes an 8-hydroxy-2-deoxy guanosine-coated plate and special HRP-conjugated antibody for detection.

Measurement of SOD, MDA, and GST

The biological activities of superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione *S*-transferase (GST) in heart supernatants were measured by corresponding kits (Jiancheng Biochemistry, Nanjing, China) and the treatment protocols were strictly carried out according to the manufacturer instructions.

Real-Time Quantitative RT-PCR

The mRNA extraction and reverse transcription of hearts were performed by using the Transzol reagent (Transgen, Beijing, China). The Quantitect SYBRGreen I PCR kit was employed for conducting real-time PCR by using the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Richmond, CA, USA). The GenBank accession numbers, sequences, annealing DNA temperatures, and special DNA fragments of all the primers used in this study are listed in Table 1. The relative quantification of the expression of the target genes was performed using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control.

Western Blot

Total protein of heart tissues was extracted by protein lysate and the protein concentration was measured according to the manufacturer's instructions of protein extraction and detection kits (Beyotime, Shanghai, China). The specific protocols of western blot of growth arrest and DNA damage inducible gene 153 (GADD153), MutT Homolog 1 (MTH1), X-ray repair cross-complementing group 1 (XRCC1), and β-actin were described in our previous study [28]. The rabbit polyclonal antibodies of GADD153, MTH1, XRCC1, and β-actin were employed at different dilution ratios of GADD153 (Sc-575, dilution ratio 1:100), MTH1 (Sc-67291, dilution ratio 1:100), XRCC1 (Sc-11429, dilution ratio 1:100) (Santa Cruz, CA, USA), and β-actin (AB10024, dilution ratio 1:3000, Sangon, Shanghai, China) staying overnight at 4 °C. Then the infrared-labeled goat anti-rabbit secondary antibody [Alexa Flor 680 goat anti-rabbit IgG (H+L) USA] was adopted with a concentration of 1:20,000 at room temperature for 1.5 h. The relative quantification of the protein expression of the target genes was performed using the housekeeping gene β-actin protein as an internal control, by using the Odyssey Infrared Imaging System (Li-COR Biosciences, USA).

Statistical Analysis

The one-way ANOVA statistical analyses were performed using the Statistical Program for Social Sciences 19.0 (SPSS 19.0) software and statistically significant differences between the groups were determined using the post-hoc comparison tests and Fisher's least significant difference (LSD) test. The correlation was evaluated by Pearson correlation coefficient (r) analysis. Positive correlation is indicated by $r > 0.8$. The level of statistical significance was accepted as $P < 0.05$.

Results

Effects of Histopathology in Rat Hearts Induced by $PM_{2.5}$, 1-NP and 9-NA

After treatment with $PM_{2.5}$ and different concentrations of 1-NP and 9-NA, 1.5 mg/kg b.w. $PM_{2.5}$, higher dosages of 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w.) and higher dosages of 9-NA (4.0×10^{-5} and 1.2×10^{-4} mg/kg b.w.) induced hyperemia and myocardial gap expansion compared with those in control (Fig. 1), showing an acute pathological damage in the hearts. Especially, 1.6×10^{-4} mg/kg b.w. 1-NP caused myocardial myofibril disorder, hyperemia, inflammatory cell infiltration in myocardium, presenting a pathological damage and inflammatory responses in hearts. No obvious pathological responses were observed in the hearts of rats in the presence of the lowest dose of 1-NP or 9-NA.

Effects of DNA Damage in Rat Hearts Induced by $PM_{2.5}$, 1-NP and 9-NA

From the results of comet assay (Fig. 2), 1-NP or 9-NA at the higher dosages caused DNA damage effects in hearts of rats. The higher the dose of 1-NP or 9-NA, the more serious DNA damage. Further, as shown in Fig. 3, we found that there was a significant increase in tail DNA

Table 1 Primer information used in real-time RT-PCR

Genes	Accession nos.	Sequences	
MTH1	NM_057120	Forward primer	5′-AGTGAAGAAATGCGCCCTCA-3′
Products	148 bp, 60 °C	Reverse primer	5′-TGAGGATGGTGTCCTGACCA-3′
GADD153	RNU30186	Forward primer	5′-GTCACAAGCACCTCCCAAAG-3′
Products	110 bp, 60 °C	Reverse primer	5′-CCACTCTGTTTCCGTTTCCT-3′
XRCC1	NM_053435	Forward primer	5′-GATGGGGAACAGTCAGAAGGAC-3′
Products	195 bp, 60 °C	Reverse primer	5′-AATTGGCAGGTCAGCCTCTG-3′
OGG1	NM_030870	Forward primer	5′-CAACATTGCTCGCATCACTGG-3′
Products	195 bp, 60 °C	Reverse primer	5′-ATGGCTTTAGCACTGGCACATACA-3′
GAPDH	NM_017008	Forward primer	5′-ATGTATCCGTTGTGGATCTGAC-3′
Products	78 bp, 56 °C	Reverse primer	5′-CCTGCTTCACCACCTTCTTG-3′

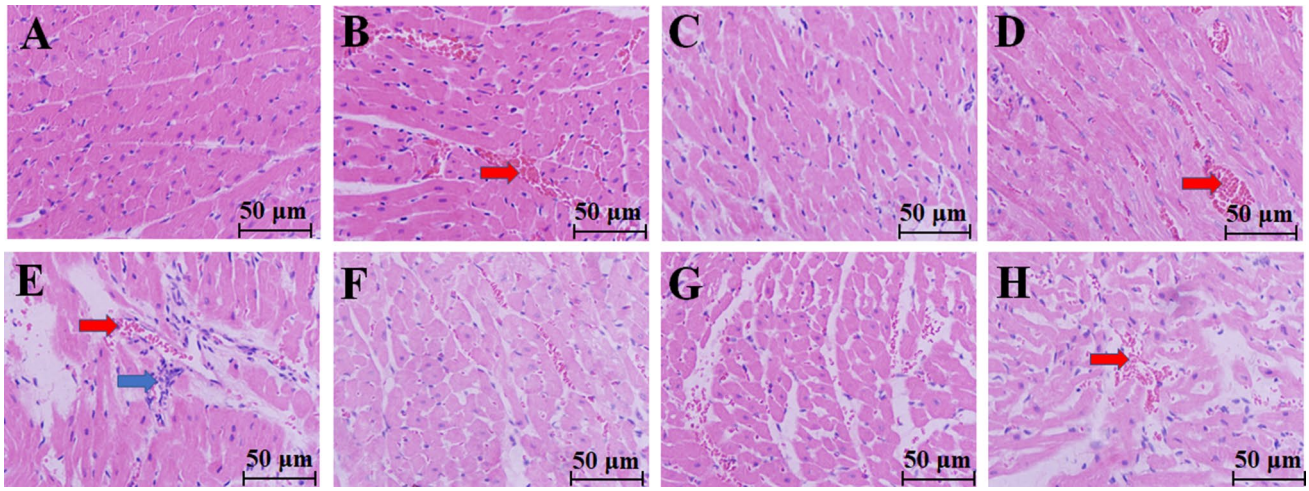

Fig. 1 The morphological characteristics in the hearts of rats from saline control (**A**), 1.5 mg/kg b.w. $PM_{2.5}$ (**B**), 1.0×10^{-5} mg/kg b.w. 1-NP (**C**), 4.0×10^{-5} mg/kg b.w. 1-NP (**D**), 1.6×10^{-4} mg/kg b.w. 1-NP (**E**), 1.3×10^{-5} mg/kg b.w. 9-NA (**F**), 4.0×10^{-5} mg/kg b.w. 9-NA (**G**) and 1.2×10^{-4} mg/kg b.w. 9-NA (**H**), ×400 magnification. The red arrows indicate sites of hyperemia, the blue arrow indicates site of inflammatory cell infiltration, respectively. (Color figure online)

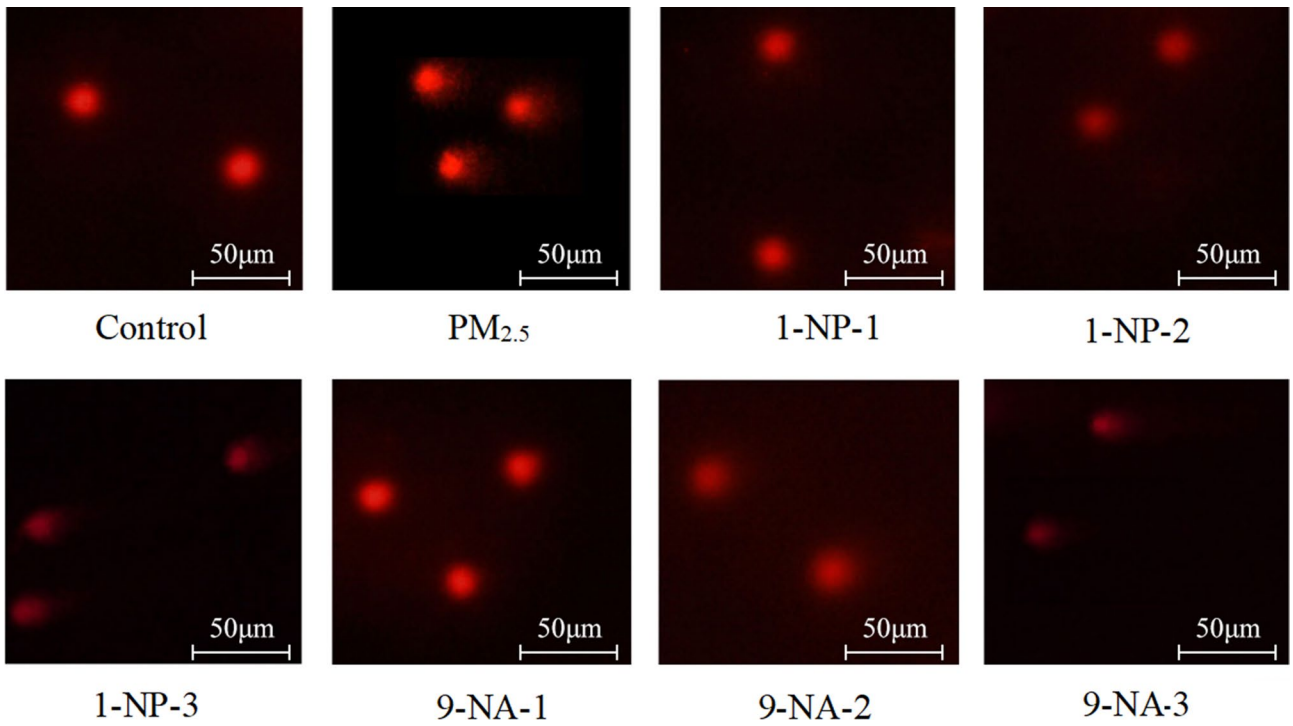

Fig. 2 Images of comet assay in the hearts of rats of different groups. ×400 Magnification

percentage (%), OTM and tail length values in rat heart cells treated with $PM_{2.5}$, 1-NP and 9-NA at all doses tested compared to the control ($P<0.05$ or <0.01). 9-NA increased OTM while 1-NP and 9-NA enhanced tail length in a dose-dependent manner (Fig. 3A, B, $r>0.8$), in which the change of tail length was significantly correlated with 9-NA dose ($r=0.963$, $P=0.037$).

DPC levels were statistically elevated in rat hearts treated with $PM_{2.5}$, three dosages of 1-NP and three dosages of 9-NA ($P<0.01$) as shown in Fig. 3C. Similarly,

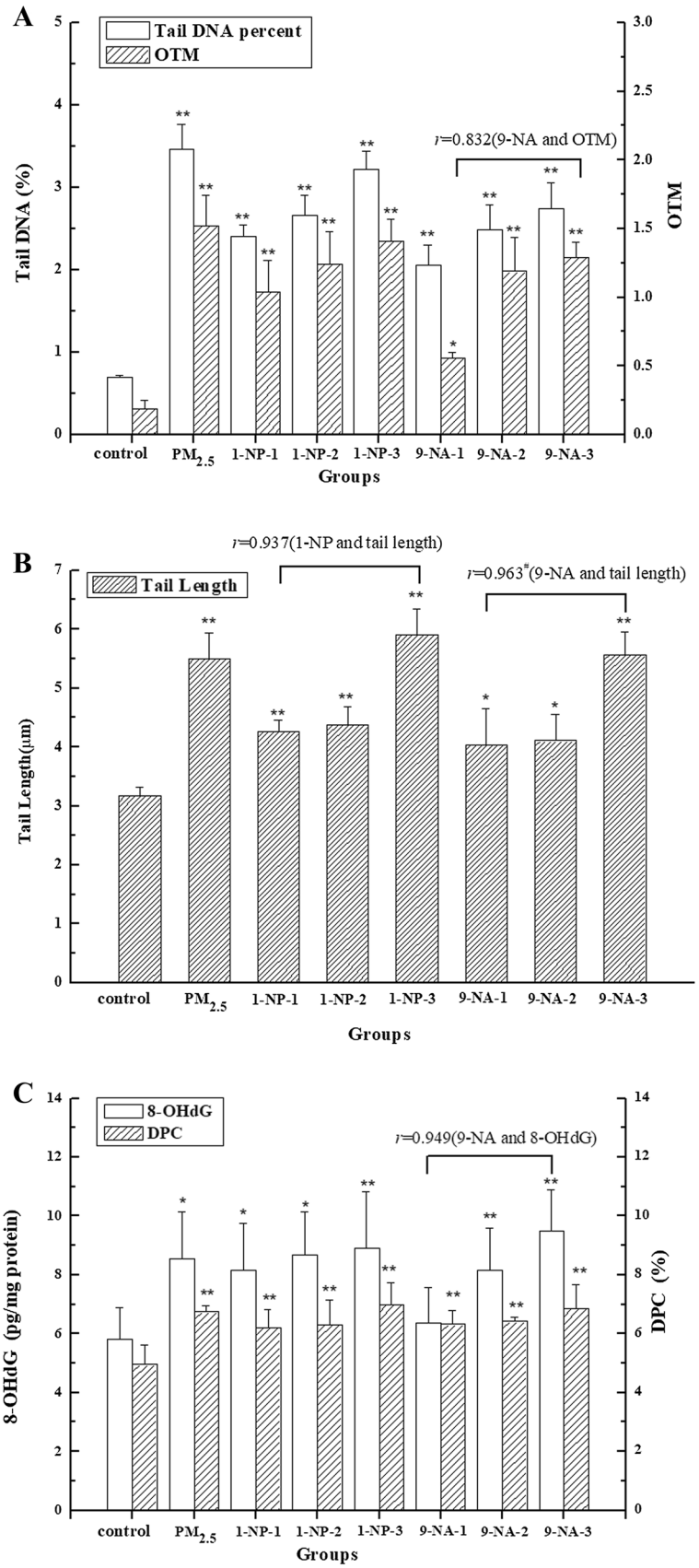

Fig. 3 DNA damage results in the hearts of rats of different groups; changes of tail DNA % and OTM (**A**), tail length (**B**) and levels of 8-OHdG and DPC (**C**). The values are mean ± SD from three individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by $^{*}P < 0.05$ and $^{**}P < 0.01$. Using Pearson correlation coefficient (r) analysis, positive correlation is indicated by $r > 0.8$, significant difference is indicated by $^{\#}P < 0.05$. Control: 5% DMSO, $PM_{2.5}$: 1.5 mg/kg b.w. $PM_{2.5}$ suspension, 1-NP-1: 1.0×10^{-5} mg/kg b.w. 1-NP solution, 1-NP-2: 4.0×10^{-5} mg/kg b.w. 1-NP solution, 1-NP-3: 1.6×10^{-4} mg/kg b.w. 1-NP solution, 9-NA-1: 1.3×10^{-5} mg/kg b.w. 9-NA solution, 9-NA-2: 4.0×10^{-5} mg/kg b.w. 9-NA solution, and 9-NA-3: 1.2×10^{-4} mg/kg b.w. 9-NA solution

the 8-OHdG levels were significantly elevated in the hearts of rats induced by $PM_{2.5}$, three dosages of 1-NP, and two higher dosages of 9-NA compared to the control ($P<0.05$ or <0.01). 9-NA elevated 8-OHdG levels in a dose-dependent manner (Fig. 3C, $r=0.949$).

Moreover, the DNA damage effects were compared and evaluated in the hearts of rats treated with $PM_{2.5}$, 1-NP and 9-NA when 1-NP (4.0×10^{-5} mg/kg b.w.) or 9-NA dosage (4.0×10^{-5} mg/kg b.w.) was comparable to dosage of 1.5 mg/kg b.w. $PM_{2.5}$ in this study. The results of comet assay showed that tail lengths were 5.50, 4.38, and 4.11 μm, tail DNA content were 3.46%, 2.66%, and 2.49%; OTM values were 1.52, 1.24, and 1.19 in the hearts in the presence of 1.5 mg/kg b.w. $PM_{2.5}$, 4.0×10^{-5} mg/kg b.w. of 1-NP, and 4.0×10^{-5} mg/kg b.w. of 9-NA. It suggested that the order of DNA strand breaks in hearts was $PM_{2.5}$ > 1-NP > 9-NA, from high to low. The results of DPC and 8-OHdG proved that the three pollutants caused similar DNA injury effects.

Effects of Oxidative Stress Markers in Rat Hearts Induced by $PM_{2.5}$, 1-NP, and 9-NA

As shown in Table 2, $PM_{2.5}$ (1.5 mg/kg b.w.) and the highest dose 9-NA (1.2×10^{-4} mg/kg b.w.) caused significant increases in the levels of GST, MDA, and HO-1 while inhibited SOD activity in the hearts of rats compared with the control ($P<0.05$ or <0.01). As for 1-NP, MDA and HO-1 levels were promoted obviously whereas SOD activity was increased in the presence of higher doses 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w.) compared to the control ($P<0.05$ or <0.01). Heart GST activity induced by 1.6×10^{-4} mg/kg b.w. 1-NP was markedly higher than that of the control ($P<0.01$). The changes of GST, HO-1, MDA, and SOD levels in the presence of lowest dose 1-NP (1.0×10^{-5} mg/kg b.w.) or lowest and higher dose 9-NA (1.3×10^{-5} and 4.0×10^{-5} mg/kg b.w.) were not statistically significant compared with the control group ($P>0.05$).

Based on the results of correlation analysis, 9-NA caused the changes of GST ($r=0.993$, $P=0.007$), HO-1 ($r=0.985$, $P=0.015$), MDA ($r=0.977$, $P=0.023$), and SOD ($r=0.987$, $P=0.013$) levels with a dose–effect relationship, respectively. Furthermore, the levels of GST ($r=0.968$, $P=0.032$) and MDA ($r=0.973$, $P=0.027$) were strongly correlated with 1-NP doses.

Effects of DNA Damage and Repair Relative Gene Expression in Rat Hearts Induced by $PM_{2.5}$, 1-NP and 9-NA

In Fig. 4A, there was a significant increase in GADD153 and OGG1 mRNA and protein levels of rat hearts exposed to $PM_{2.5}$, higher dosages 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w.) and higher dosages 9-NA (4.0×10^{-5} and 1.2×10^{-4} mg/kg b.w.) compared to the control ($P<0.05$ or <0.01). Also, the protein levels of OGG1 in hearts in the presence of 1.0×10^{-5} mg/kg b.w. 1-NP and 1.3×10^{-5} mg/kg b.w. 9-NA were obviously higher than that of the control ($P<0.01$). The mRNA and protein expressions of GADD153 as well as the OGG1 mRNA in rats treated with 1.0×10^{-5} mg/kg b.w. 1-NP or 1.3×10^{-5} mg/kg b.w. 9-NA were not significantly changed relative to the control ($P>0.05$). 1-NP or 9-NA enhanced OGG1 expression in a concentration-dependent manner ($r>0.84$); 1-NP increased GADD153 protein levels showing a dose–effect relationship ($r>0.80$).

In Fig. 5A, the DNA damage repair gene MTH1 and XRCC1 mRNA and protein levels were suppressed obviously in the presence of $PM_{2.5}$ and higher doses 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w.) compared to the control ($P<0.05$ or <0.01). As for 9-NA, the highest dosage

Table 2 SOD, GST, and HO-1 activities and MDA contents in hearts treated with $PM_{2.5}$, 1-NP, or 9-NA

Groups	SOD (U/mg prot)	GST (U/mg prot)	MDA (nmol/mg prot)	HO-1 (ng/mg prot)
Control	38.0 ± 3.8	23.4 ± 3.8	1.06 ± 0.11	33.6 ± 5.1
$PM_{2.5}$ (1.5×10^{-5} mg/kg b.w.)	32.0 ± 3.8*	32.5 ± 4.4*	1.34 ± 0.18*	44.9 ± 6.0**
1-NP-1 (1.0×10^{-5} mg/kg b.w.)	34.3 ± 2.4	26.3 ± 6.1	1.21 ± 0.25	37.0 ± 5.3
1-NP-2 (4.0×10^{-5} mg/kg b.w.)	32.3 ± 5.7*	28.5 ± 8.1	1.35 ± 0.20*	41.6 ± 6.0*
1-NP-3 (1.6×10^{-4} mg/kg b.w.)	30.0 ± 5.5**	34.6 ± 7.0**	1.70 ± 0.23**	46.6 ± 6.5**
1-NP dose–effect relationship (r)	−0.843	0.986#	0.973#	0.926
9-NA-1 (1.3×10^{-5} mg/kg b.w.)	36.4 ± 3.7	25.0 ± 7.1	1.12 ± 0.24	35.5 ± 5.2
9-NA-2 (4.0×10^{-5} mg/kg b.w.)	35.5 ± 2.6	27.4 ± 5.4	1.24 ± 0.20	39.3 ± 5.2
9-NA-3 (1.2×10^{-4} mg/kg b.w.)	32.1 ± 3.4*	32.6 ± 6.4*	1.40 ± 0.05*	45.0 ± 5.3**
9-NA dose–effect relationship (r)	−0.987#	0.993##	0.977#	0.985#

The values are mean ± SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by $^{*}P<0.05$ and $^{**}P<0.01$. Using Pearson correlation coefficient (r) analysis, positive correlation is indicated by $r>0.8$, significant difference is indicated by $^{\#}P<0.05$ and $^{\#\#}P<0.01$

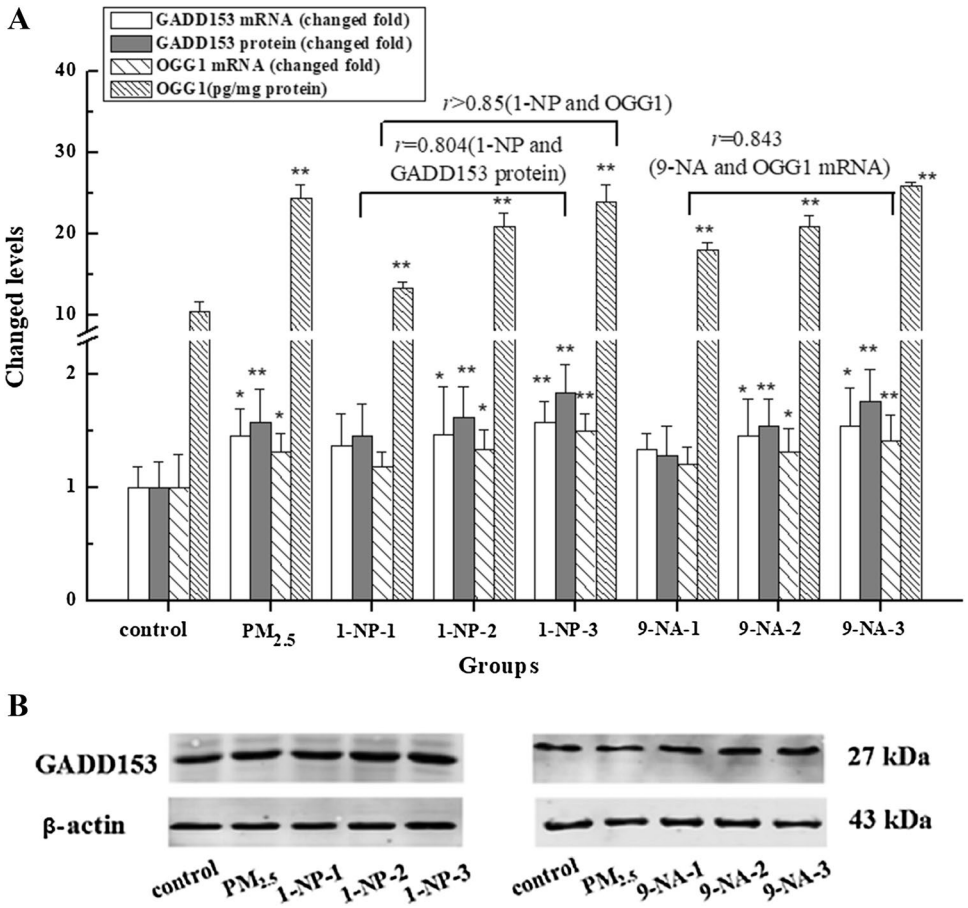

Fig. 4 Expression of mRNA and protein of GADD153 and OGG1 in rat hearts treated with $PM_{2.5}$, 1-NP or 9-NA (**A**), and protein bands (**B**). The values are mean ± SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated $*P<0.05$ and $**P<0.01$. Using Pearson correlation coefficient (r) analysis, positive correlation is indicated by $r>0.8$. The grouping in the figure is the same as the Fig. 3

pollutant (1.2×10^{-4} mg/kg b.w.) significantly inhibited MTH1 and XRCC1 mRNA and protein expression in rat hearts compared with the control ($P<0.05$ or <0.01). The higher dosage 9-NA (4.0×10^{-5} mg/kg b.w.) markedly decreased MTH1 protein expression in the hearts relative to the control ($P<0.05$). No significant changes of the MTH1 and XRCC1 gene expression were observed in the rats exposed to the lowest dosage of 1-NP or 9-NA, versus the control ($P>0.05$). 1-NP or 9-NA decreased MTH1 and XRCC1 expression levels showing a dose–effect relationship ($|r|>0.82$); a significant correlation was apparent between 9-NA doses and MTH1 mRNA expression ($r=-0.986$, $P=0.014$).

The images of representative protein bands of GADD153, MTH1, XRCC1, and β-actin in western blot were shown in Figs. 4B and 5B.

Comparison of Heart DNA Damage Effects in Rats Exposed to $PM_{2.5}$ (1.5 mg/kg b.w.), 1-NP (4.0×10^{-5} mg/kg b.w.), or 9-NA (4.0×10^{-5} mg/kg b.w.)

To compare the relevance in DNA damage induced by $PM_{2.5}$ (1.5 mg/kg b.w.) or higher dosage 1-NP (4.0×10^{-5} mg/kg b.w.) or higher dosage 9-NA (4.0×10^{-5} mg/kg b.w.), the correlations of three DNA damage markers including DNA tail length, 8-OHdG and DPC with $PM_{2.5}$, 1-NP (4.0×10^{-5} mg/kg b.w.) or 9-NA (4.0×10^{-5} mg/kg b.w.) were analyzed. The results showed that $PM_{2.5}$, 1-NP or 9-NA respectively induced an increase in the DNA damage levels (Table 3). $PM_{2.5}$ and 1-NP exposure had significantly correlation in DPC levels ($r=0.997$, $P=0.047$). The correlation of DNA tail length or DPC levels between $PM_{2.5}$ and 9-NA was significant (for tail length: $r=0.999$, $P=0.03$; DPC levels: $r=0.999$, $P=0.022$).

Discussion

Ambient $PM_{2.5}$ presents an air exposure hazard and is a complex mixture of different chemicals like metals, PAHs and NPAHs, which is suggested to be a key determinant of the adverse health effects. Epidemiological studies had confirmed that $PM_{2.5}$ exposure increased the risks of incidence and mortality of heart failure and myocardial infarction [36, 37]. Exposures to ambient $PM_{2.5}$ were associated with elevated resting heart rate and blood pressure [38, 39]. Of note, $PM_{2.5}$ exposure significantly increased heart rate in a general

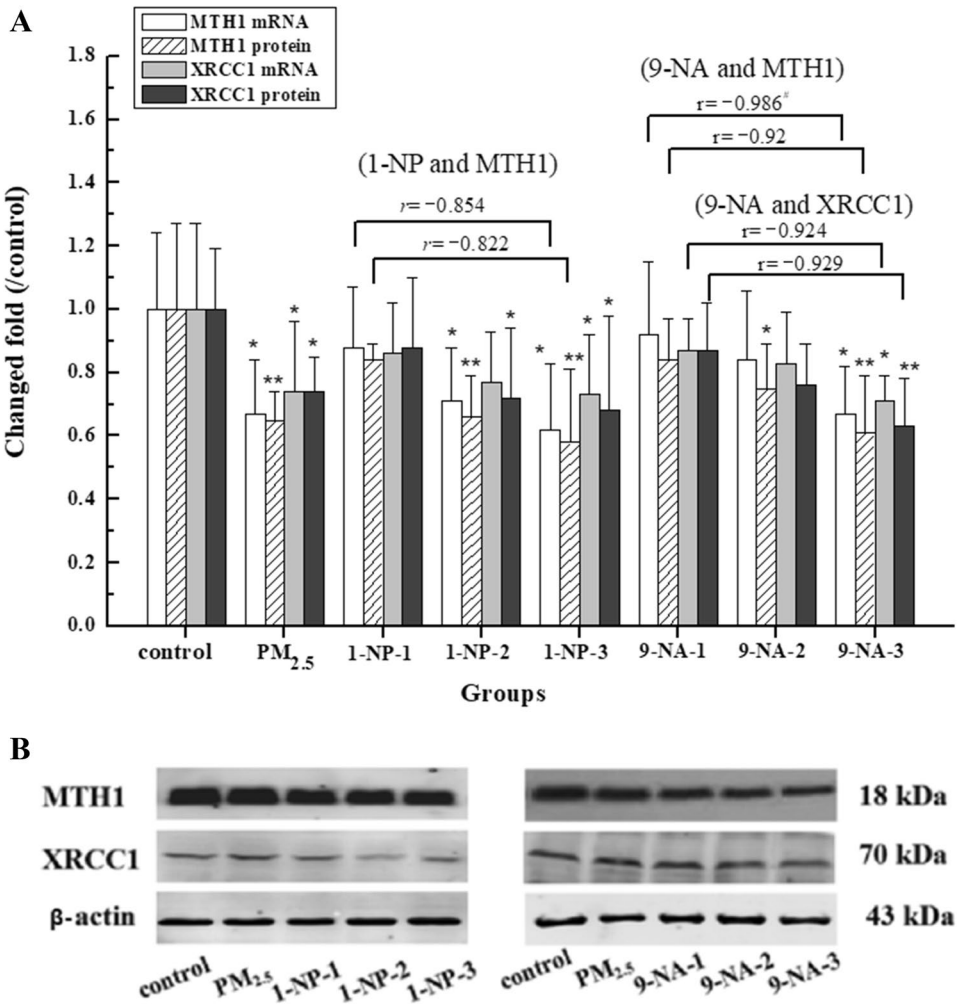

Fig. 5 Expression of mRNA and protein of MTH1 and XRCC1 in rat hearts treated with $PM_{2.5}$, 1-NP or 9-NA (**A**), and protein bands (**B**). The values are mean ± SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated $^{*}P < 0.05$ and $^{**}P < 0.01$. Using Pearson correlation coefficient (r) analysis, positive correlation is indicated by $r > 0.8$, significant difference is indicated by $^{\#}P < 0.05$. The grouping in the figure is the same as the Fig. 3

Table 3 Comparison of heart DNA damage effects in rats exposed to $PM_{2.5}$ (1.5 mg/kg b.w.), 1-NP (4.0×10^{-5} mg/kg b.w.), or 9-NA (4.0×10^{-5} mg/kg b.w.)

Index	r	
	$PM_{2.5}$ to 1-NP-2	$PM_{2.5}$ to 9-NA-2
Tail length (μm)	0.969	$0.999^{\#}$
8-OHdG (pg/mg prot)	0.966	0.997
DPC (%)	$0.997^{\#}$	$0.999^{\#}$

Using Pearson correlation coefficient (r) analysis, positive correlation is indicated by $r > 0.8$, significant difference is indicated by $^{\#}P < 0.05$

adult population along with elevated urinary 8-OHdG, a biomarker of DNA oxidative stress [40]. These data suggest that the adverse cardiac effects from $PM_{2.5}$ pollution are more susceptible to individuals who suffer excessive DNA oxidative damage. However, the precise mechanisms of $PM_{2.5}$ on DNA injury in the hearts remain obscure. In current study, our results further revealed that the histopathological changes and DNA damage were mediated by DNA repair genes and oxidative stress induced by $PM_{2.5}$, 1-NP or 9-NA in the hearts of rats, considering carcinogenicity of $PM_{2.5}$ and 1-NP [1, 12] and weak mutagenicity of 9-NA [17].

Previous studies indicated that $PM_{2.5}$ exposure increased DNA strand breaks in human peripheral blood lymphocytes and 8-OHdG levels in human urinary [40, 41], and that $PM_{2.5}$ or 1-NP/9-NA caused heart DNA damage in rat lungs, human bronchial epithelial cells or endothelial cells, and rat alveolar macrophages [13, 24, 28, 42, 43]. However, the data of $PM_{2.5}$ or 1-NP/9-NA-induced heart DNA damage are limited so far. The present study found that $PM_{2.5}$, 1-NP and 9-NA caused DNA strand breaks, DPC formation and 8-OHdG level elevation in the hearts of rats, and led to heart injury, which was mediated by oxidative stress and DNA damage and repair gene. To the best of our knowledge, this is the first report on rat heart DNA damage effects and regulation mechanism of $PM_{2.5}$, 1-NP and 9-NA in a typically polluted areas, China.

Firstly, oxidative damage mediated by ROS can be one of the main mechanisms of DNA damage [44]. ROS can interact with the DNA molecule and cause several types of DNA

damage including DNA strand breaks, modification of DNA bases, 8-OHdG formation, DNA–protein cross-linkage, and damage to the DNA repair system. The research revealed that DE particles increased ROS and lipid peroxidation levels and induced DNA strand breaks in the heart of mice [45]. Further, the studies revealed the pollutants-produced excess ROS induced oxidative stress, which mediated DNA damage in mouse hearts [46]. Some ROS inhibitors and antioxidative enzymes or antioxidants pre-treatment effectively attenuated the pollutant-induced ROS generation, inhibiting DNA damage [46–48]. The up-regulated expression of antioxidant enzyme genes like catalase, glutathione peroxidase and SOD may reduce the levels of intracellular ROS and DNA damage in the cells [48]. For example, SOD may catalyze the dismutation of the superoxide radical ($O_2^{\bullet-}$) into hydrogen peroxide and molecular oxygen, while GST may scavenge hydrogen peroxide and reduce the oxidation of organic hydrogen, quenching of ROS [49]. HO-1 is the rate-limiting enzyme of heme degradation and prevents the accumulation of free heme in cell membranes, alleviating heme-induced oxidative stress [50]. HO-1 is also an inducible antioxidant enzyme and may be activated when oxidative stress occurs in the cells under the oxide stimulus. Generally, the delicate balance between ROS and antioxidant defense system affects the development of oxidative stress. When excessive ROS attack the lipids in cytomembrane, it would trigger lipid peroxidation reaction and the accumulation of MDA (a typical lipid peroxidation product); when ROS attack DNA molecules, it would induce DNA strand damage and DNA oxidation damage (8-OHdG production). When ROS attack protein molecules, it would result in protein oxidative damage and DPCs [51, 52]. $PM_{2.5}$ had been proven to generate ROS, such as $O_2^{\bullet-}$ and $^{\bullet}OH$ and may trigger potential oxidative damage [53]. Our results (Figs. 2, 3; Table 2) observed that $PM_{2.5}$ induced heart pathological injury and DNA damage (DNA strand breakages and the increases of 8-OHdG and DPC levels). In this process, the pollutants inhibited $O_2^{\bullet-}$ scavenging activities of SOD activities and enhanced the levels of MDA, inducing lipid peroxidation, one of oxidative stress responses. Increased GST and HO-1 activity can be interpreted as an adaptive response to oxidative stress. The results indicated that the intensified oxidative stress not only triggered myocardial pathological injury and mediated DNA damage in heart of rats exposed to $PM_{2.5}$. 1-NP and 9-NA had similar effects on pathological injury, DNA damage, and oxidative stress in the hearts of rats. It was reported that 1-NP increased intracellular ROS levels and induce DNA damage and 8-OHdG formation [13, 54], and 1-NP and 9-NA caused lung DNA damage and oxidative stress [24, 28], which are consistence with our results.

Secondly, DNA damage susceptibility genes and DNA repair genes play a key role in the regulation of the genotoxic effect of DNA damaging agents. On the one hand, GADD153, a DNA damage susceptibility gene, can be highly promoted in the process of DNA damage initiated by the genotoxic agents [55]. For example, quinone thioether caused DNA single-strand breaks and increase the expression of GADD153 in renal proximal tubular epithelial cells via a ROS regulation pathway [56]. DNA damage in the lungs of rats exposed to $PM_{2.5}$ and some NPAHs provided the initial signal for GADD153 activation [24, 28]. In line with the above reports, our data showed that $PM_{2.5}$, 1-NP, or 9-NA caused DNA injury and up-regulated GADD153 gene in hearts of rats, indicating that DNA is sensitive to $PM_{2.5}$, 1-NP or 9-NA, and is prone to damage. On the other hand, DNA repair genes play a critical role in protecting the human DNA from damage caused by potent carcinogens in the environment [31–33, 57, 58]. Among DNA repair genes, OGG1 is a base excision repair (BER) enzyme, which may excise 8-oxo-7,8-dihydro-2-deoxyguanine (8-oxoG), a pro-mutagenic lesion induced by oxidative stress [57]. OGG1 deficiency increased the susceptibly to particles and exacerbated the DNA damage effect [57]. MTH1, another BER enzyme, may effectively hydrolyze 8-oxo-dGTP to 8-oxodGMP, and then prevent 8-oxo-dGTP from incorporating into DNA molecules [32]. Additionally, XRCC1 acts as a scaffolding protein in the converging BER and single-strand break repair (SSBR) pathways [58]. If DNA repair genes were inhibited and unable to effectively exert DNA repair roles, DNA damage such as DNA strand breaks, 8-OHdG formation along with the unrepaired or mispairing bases would occur, then leading to cell genotoxicity or possibly disease. Based on our results, higher expression of OGG1 in rat hearts is helpful for enhancing the capability of removing 8-oxoG, whereas the decreases of MTH1 and XRCC1 gene expression may impair BER and SSBR repair, and ultimately leading to DNA strand breaks and 8-OHdG formation. Given heart DNA damage happened, it may be speculated that inhibition ability of MTH1 and XRCC1 in hearts incurred by $PM_{2.5}$, 1-NP or 9-NA is higher than enhancement ability of OGG1. These results suggest a novel mechanism of $PM_{2.5}$ and NPAHs-caused heart DNA damage, in which $PM_{2.5}$ and NPAHs increase the susceptibility to heart DNA damage through enhancing GADD153 expression and augment the risks of heart DNA injury through special DNA repair signaling mechanisms involved in OGG1, MTH1, and XRCC1. Given the complexity of DNA repair roles, the underlying molecular mechanisms of $PM_{2.5}$ and NPAHs-induced heart genotoxicity need to be deeply investigated.

Finally, we focused on the correlation comparison of heart DNA damage effects in rats exposed to $PM_{2.5}$ (1.5 mg/kg b.w.) and 1-NP (4.0×10^{-5} mg/kg b.w.) or 9-NA (4.0×10^{-5} mg/kg b.w.) under the approximately equivalent dose levels. Combined with the data in Fig. 3, the sequence of DNA damage degrees (DNA tail length, tail DNA content, OTM and DPC) from high to low was

as follows: $PM_{2.5}$ > 1-NP (4.0×10^{-5} mg/kg b.w.) ≈ 9-NA (4.0×10^{-5} mg/kg b.w.). The numerical values of 8-OHdG levels in rat hearts exposed to $PM_{2.5}$, 4.0×10^{-5} mg/kg b.w. 1-NP or 4.0×10^{-5} mg/kg b.w. 9-NA were similar. It hinted that $PM_{2.5}$-caused heart toxicity was higher than that of 1-NP or 9-NA under the experimental conditions, which may be caused by the complex chemical compositions of $PM_{2.5}$, especially toxic organic matters. At the same time, we noticed that the increases of OTM, tail length and DPC levels between $PM_{2.5}$ and 1-NP or 9-NA exposure had positive correlations, and the values of *r* were more than 0.8. We hypothesize that 1-NP or 9-NA in $PM_{2.5}$ may relate to $PM_{2.5}$-induced heart DNA damage. Oh et al. [59] reported that the effects of OTM of NPAH fractionated extracts in BEAS-2B cells were lower than that of crude extract of $PM_{2.5}$ and NPAH extracts were the biologically active fractions of $PM_{2.5}$ for the genotoxic effects [59], which is consistent with our results above. Further studies about the contributions of NPAHs to $PM_{2.5}$-induced genotoxicity in the heart are very conducive to understand the toxicology mechanisms for $PM_{2.5}$ cardiac toxicity.

Conclusions

(1) $PM_{2.5}$, 1-NP, and 9-NA under the experimental conditions caused DNA damage effects involved in DNA strand breaks, 8-OHdG formation and DPC increase.
(2) $PM_{2.5}$, 1-NP, and 9-NA significantly promote oxidative stress accompanied by the increases of GST, HO-1, and MDA levels and the suppression of SOD activity.
(3) $PM_{2.5}$, 1-NP, and 9-NA significantly activated DNA damage susceptibility gene GADD153 and decreased the DNA repair gene MTH1 and XRCC1 expression, such inhibition effects exceeded the scavenging role of OGG1 to damaged DNA.
(4) The heart DNA injury effect induced by $PM_{2.5}$ was greater than that of 1-NP or 9-NA under the comparable exposure levels, and $PM_{2.5}$-induced DNA damage had a marked positive correlation with 1-NP or 9-NA, which implies that $PM_{2.5}$-bound 1-NP or 9-NA may account for some of the DNA damage caused by $PM_{2.5}$.

Acknowledgements This research was supported by the National Natural Science Foundation of China (No. 91543202) and the Nature Science Foundation of Shanxi Province in China (No. 2014011036-2).

References

1. IARC. (2016). Outdoor air pollution. In *Monographs on the evaluation of carcinogenic risks to humans* (Vol. 109). Lyon: IARC.
2. Dominici, F., Peng, R. D., Bell, M. L., Pham, L., McDermot, A., Zeger, S. L., et al. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. *JAMA, 295*(10), 1127–1134.
3. Xu, Q., Wang, S., Guo, Y., Wang, C., Huang, F., Li, X., et al. (2017). Acute exposure to fine particulate matter and cardiovascular hospital emergency room visits in Beijing, China. *Environmental Pollution, 220*(Pt A), 317–327.
4. Thurston, G. D., Burnett, R. T., Turner, M. C., Shi, Y., Krewski, D., Lall, R., et al. (2016). Ischemic heart disease mortality and long-term exposure to source-related components of U.S. fine particle air pollution. *Environmental Health Perspectives, 124*(6), 785–794.
5. Fu, P. P., & Herreno-Saenz, D. (1999). Nitro-polycyclic aromatic hydrocarbons: A class of genotoxic environmental pollutants. *Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis and Ecotoxicology Reviews, 17*, 1–43.
6. Yang, X., Igarashi, K., Tang, N., Lin, J. M., Wang, W., Kameda, T., et al. (2010). Indirect- and direct-acting mutagenicity of diesel, coal and wood burning-derived particulates and contribution of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons. *Mutation Research, 695*, 29–34.
7. IARC. (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. In *Monographs on the evaluation of carcinogenic risks to humans* (Vol. 92). Lyon: IARC.
8. Wang, W., Jariyasopit, N., Schrlau, J., Jia, Y., Tao, S., Yu, T. W., et al. (2011). Concentration and photochemistry of PAHs, NPAHs, and OPAHs and toxicity of $PM_{2.5}$ during the Beijing Olympic Games. *Environmental Science and Technology, 45*, 6887–6895.
9. Hayakawa, K. (2016). Environmental behaviors and toxicities of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons. *Chemical and Pharmaceutical Bulletin (Tokyo), 64*, 83–94.
10. Jiang, P., Yang, L., Chen, X., Gao, Y., Li, Y., Zhang, J., et al. (2018). Impact of dust storms on NPAHs and OPAHs in $PM_{2.5}$ in Jinan, China, in spring 2016: Concentrations, health risks, and sources. *Aerosol and Air Quality Research, 18*, 471–484.
11. Lin, Y., Ma, Y., Qiu, X., Li, R., Fang, Y., & Wang, J., et al. (2015). Sources, transformation, and health implications of PAHs and their nitrated, hydroxylated, and oxygenated derivatives in $PM_{2.5}$ in Beijing. *Journal of Geophysical Research Atmospheres, 120*(14), 7219–7228.
12. IARC. (1989). Diesel and gasoline engine exhausts and some nitroarenes. In *Monographs on the evaluation of carcinogenic risks to humans* (Vol. 46). Lyon: IARC.
13. Andersson, H., Piras, E., Demma, J., Hellman, B., & Brittebo, E. (2009). Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells. *Toxicology, 262*(1), 57–64.
14. Wilson, S. J., Miller, M. R., & Newby, D. E. (2018). Effects of diesel exhaust on cardiovascular function and oxidative stress. *Antioxidants and Redox Signaling, 28*(9), 819–836.
15. Miller-Schulze, J. P., Paulsen, M., Kameda, T., Toriba, A., Tang, N., Tamura, K., et al. (2013). Evaluation of urinary metabolites of 1-nitropyrene as biomarkers for exposure to diesel exhaust in taxi drivers of Shenyang, China. *Journal of Exposure Science and Environmental Epidemiology, 23*(2), 170–175.
16. Zhang, Y., Li, R., Fang, J., Wang, C., & Cai, Z. (2018). Simultaneous determination of eighteen nitro-polyaromatic hydrocarbons in $PM_{2.5}$ by atmospheric pressure gas chromatography–tandem mass spectrometry. *Chemosphere, 198*, 303–310.
17. Fu, P. P., Heflich, R. H., Von Tungeln, L. S., Yang, D. T., Fifer, E. K., & Beland, F. A. (1986). Effect of the nitro group conformation on the rat liver microsomal metabolism and bacterial mutagenicity of 2- and 9-nitroanthracene. *Carcinogenesis, 7*(11), 1819–1827.

18. Feng, S., Gao, D., Liao, F., Zhou, F., & Wang, X. (2016). The health effects of ambient $PM_{2.5}$ and potential mechanisms. *Ecotoxicology and Environmental Safety, 128*, 67–74.
19. Yang, X., Feng, L., Zhang, Y., Hu, H., Shi, Y., Liang, S., et al. (2018). Cytotoxicity induced by fine particulate matter ($PM_{2.5}$) via mitochondria-mediated apoptosis pathway in human cardiomyocytes. *Ecotoxicology and Environmental Safety, 161*, 198–207.
20. Li, R., Kou, X., Geng, H., Xie, J., Tian, J., Cai, Z., et al. (2015). Mitochondrial damage: An important mechanism of ambient $PM_{2.5}$ exposure-induced acute heart injury in rats. *Journal of Hazardous Materials, 287*, 392–401.
21. Liu, D., Lin, T., Syed, L. T., Cheng, Z., Xu, Y., Li, K., Zhang, G., et al (2017). Concentration, source identification, and exposure risk assessment of $PM_{2.5}$-bound parent PAHs and nitro-PAHs in atmosphere from typical Chinese cities. *Scientific Reports, 7*(1), 10398.
22. Andersson, J. T., & Achten, C. (2015). Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. *Polycyclic Aromatic Compounds, 35*(2–4), 330–354.
23. Mayati, A., Le Ferrec, E., Holme, J. A., Fardel, O., Lagadic-Gossmann, D., & Ovrevik, J. (2014). Calcium signaling and β2-adrenergic receptors regulate 1-nitropyrene induced CXCL8 responses in BEAS-2B cells. *Toxicology In Vitro, 28*(6), 1153–1157.
24. Li, R., Zhao, L., Zhang, L., Chen, M., Dong, C., & Cai, Z. (2017). DNA damage and repair, oxidative stress and metabolism biomarker responses in lungs of rats exposed to ambient atmospheric 1-nitropyrene. *Environmental Toxicology and Pharmacology, 54*, 14–20.
25. Shang, Y., Zhou, Q., Wang, T., Jiang, Y., Zhong, Y., Qian, G., et al. (2017). Airborne nitro-PAHs induce Nrf2/ARE defense system against oxidative stress and promote inflammatory process by activating PI3K/Akt pathway in A549 cells. *Toxicology In Vitro, 44*, 66–73.
26. Øvrevik, J., Holme, J. A., Låg, M., Schwarze, P. E., & Refsnes, M. (2013). Differential chemokine induction by 1-nitropyrene and 1-aminopyrene in bronchial epithelial cells: Importance of the TACE/TGF-α/EGFR-pathway. *Environmental Toxicology and Pharmacology, 35*(2), 235–239.
27. Podechard, N., Tekpli, X., Catheline, D., Holme, J. A., Rioux, V., Legrand, P., et al. (2011). Mechanisms involved in lipid accumulation and apoptosis induced by 1-nitropyrene in Hepa1c1c7 cells. *Toxicology Letters, 206*(3), 289–299.
28. Li, R., Zhao, L., Zhang, L., Chen, M., Shi, J., Dong, C., et al. (2017). Effects of ambient $PM_{2.5}$ and 9-nitroanthracene on DNA damage and repair, oxidative stress and metabolic enzymes in lungs of rats. *Toxicology Research, 6*(5), 654–663.
29. Barzilai, A., & Yamamoto, K. (2004). DNA damage responses to oxidative stress. *DNA Repair, 3*(8–9), 1109–1115.
30. Bhat, M. A., & Gandhi, G. (2018). Elevated oxidative DNA damage in patients with coronary artery disease and its association with oxidative stress biomarkers. *Acta cardiologica*. https://doi.org/10.1080/00015385.2018.1475093.
31. Karahalil, B., Kesimci, E., Emerce, E., Gumus, T., & Kanbak, O. (2011). The impact of OGG1, MTH1 and MnSOD gene polymorphisms on 8-hydroxy-2′-deoxyguanosine and cellular superoxide dismutase activity in myocardial ischemia-reperfusion. *Molecular Biology Reports, 38*(4), 2427–2435.
32. Papeo, G. (2016). MutT Homolog 1 (MTH1): The silencing of a target. *Journal of Medicinal Chemistry, 59*(6), 2343–2345.
33. Risom, L., Møller, P., & Loft, S. (2005). Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research, 592*(1–2), 119–137.
34. Cao, L. X., Geng, H., Yao, C. T., Zhao, L., Duan, P. L., et al. (2014). Investigation of chemical compositions of atmospheric fine particles during a wintertime haze episode in Taiyuan City. *China Environmental Science, 34*(4), 837–843.
35. Xie, J., Fan, R., & Meng, Z. (2007). Protein oxidation and DNA–protein crosslink induced by sulfur dioxide in lungs livers, and hearts from mice. *Inhalation Toxicology, 19*, 759–765.
36. Shah, A. S., Langrish, J. P., Nair, H., McAllister, D. A., Hunter, A. L., Donaldson, K., et al. (2013). Global association of air pollution and heart failure: A systematic review and meta-analysis. *Lancet, 382*(9897), 1039–1048.
37. Mustafic, H., Jabre, P., Caussin, C., Murad, M. H., Escolano, S., Tafflet, M., et al. (2012). Main air pollutants and myocardial infarction: A systematic review and meta-analysis. *JAMA, 307*(7), 713–721.
38. Liang, R., Zhang, B., Zhao, X., Ruan, Y., Lian, H., & Fan, Z. (2014). Effect of exposure to $PM_{2.5}$ on blood pressure: A systematic review and meta-analysis. *Journal of Hypertension, 32*(11), 2130–2140.
39. Xie, X., Wang, Y., Yang, Y., Xu, J., Zhang, Y., Tang, W., et al. (2018). Long-term exposure to fine particulate matter and tachycardia and heart rate: Results from 10 million reproductive-age adults in China. *Environmental Pollution, 242*(Pt B), 1371–1378.
40. Lee, M. S., Eum, K. D., Fang, S. C., Rodrigues, E. G., Modest, G. A., & Christiani, D. C. (2014). Oxidative stress and systemic inflammation as modifiers of cardiac autonomic responses to particulate air pollution. *International Journal of Cardiology, 176*(1), 166–170.
41. Chu, M., Sun, C., Chen, W., Jin, G., Gong, J., Zhu, M., et al. (2015). Personal exposure to $PM_{2.5}$, genetic variants and DNA damage: A multi-center population-based study in Chinese. *Toxicology Letters, 235*(3), 172–178.
42. Wu, J., Shi, Y., Asweto, C. O., Feng, L., Yang, X., Zhang, Y., et al. (2017). Fine particle matters induce DNA damage and G2/M cell cycle arrest in human bronchial epithelial BEAS-2B cells. *Environmental Science and Pollution Research, 24*(32), 25071–25081.
43. Meng, Z., & Zhang, Q. (2007). Damage effects of dust storm $PM_{2.5}$ on DNA in alveolar macrophages and lung cells of rats. *Food and Chemical Toxicology, 45*(8), 1368–1374.
44. Cakmakoglu, B., Aydin, M., & Cincin, Z. B. (2011). Effect of oxidative stress on DNA repairing genes, *INTECH Open Access Publisher*. https://doi.org/10.5772/21054.
45. Nemmar, A., Beegam, S., Yuvaraju, P., Yasin, J., Tariq, S., Attoub, S., et al. (2016). Ultrasmall superparamagnetic iron oxide nanoparticles acutely promote thrombosis and cardiac oxidative stress and DNA damage in mice. *Particle and Fibre Toxicology, 13*(1), 22.
46. Zhang, P., Yi, L. H., Meng, G. Y., Zhang, H. Y., Sun, H. H., & Cui, L. Q. (2017). Apelin-13 attenuates cisplatin-induced cardiotoxicity through inhibition of ROS-mediated DNA damage and regulation of MAPKs and AKT pathways. *Free Radical Research, 51*(5), 449–459.
47. Bai, Y., Jiang, L. P., Liu, X. F., Wang, D., Yang, G., Geng, C. Y., et al. (2015). The role of oxidative stress in citreoviridin-induced DNA damage in human liver-derived HepG2 cells. *Environmental Toxicology, 30*(5), 530–537.
48. Tokarz, P., Kaarniranta, K., & Blasiak, J. (2016). Inhibition of DNA methyltransferase or histone deacetylase protects retinal pigment epithelial cells from DNA damage induced by oxidative stress by the stimulation of antioxidant enzymes. *European Journal of Pharmacology, 776*, 167–175.
49. Sharma, R., Yang, Y., Sharma, A., Awasthi, S., & Awasthi, Y. C. (2004). Antioxidant role of glutathione *S*-transferases: Protection against oxidant toxicity and regulation of stress-mediated apoptosis. *Antioxidants and Redox Signaling, 6*(2), 289–300.
50. Takahashi, T., Morita, K., Akagi, R., & Sassa, S. (2004). Heme oxygenase-1: A novel therapeutic target in oxidative tissue injuries. *Current Medicinal Chemistry, 11*(12), 1545–1561.

51. Hwang, E. S., & Kim, G. H. (2007). Biomarkers for oxidative stress status of DNA, lipids, and proteins in vitro and in vivo cancer research. *Toxicology, 229*(1–2), 1–10.
52. Arnold Groehler, I. V., Kren, S., Li, Q., Robledo-Villafane, M., Schmidt, J., Garry, M., et al. (2018). Oxidative cross-linking of proteins to DNA following ischemia-reperfusion injury. *Free Radical Biology and Medicine, 120*, 89–101.
53. Sagai, M., Saito, H., Ichinose, T., Kodama, M., & Mori, Y. (1993). Biological effects of diesel exhaust particles. I. In vitro production of superoxide and in vivo toxicity in mouse. *Free Radical Biology and Medicine, 14*(1), 37–47.
54. Kim, Y. D., Ko, Y. J., Kawamoto, T., & Kim, H. (2005). The effects of 1-nitropyrene on oxidative DNA damage and expression of DNA repair enzymes. *Journal of Occupational Health, 47*, 261–266.
55. Luethy, J. D., & Holbrook, N. J. (1992). Activation of the GADD153 promoter by genotoxic agents: A rapid and specific response to DNA damage. *Cancer Research, 52*(1), 5–10.
56. Jeong, J. K., Stevens, J. L., Lau, S. S., & Monks, T. J. (1996). Quinone thioether-mediated DNA damage, growth arrest, and gadd153 expression in renal proximal tubular epithelial cells. *Molecular Pharmacology, 50*(3), 592–598.
57. Nallanthighal, S., Chan, C., Murray, T. M., Mosier, A. P., Cady, N. C., & Reliene, R. (2017). Differential effects of silver nanoparticles on DNA damage and DNA repair gene expression in OGG1-deficient and wild type mice. *Nanotoxicology, 11*(8), 1–16.
58. Hanssen-Bauer, A., Solvang-Garten, K., Akbari, M., & Otterlei, M. (2012). X-ray repair cross complementing protein 1 in base excision repair. *International Journal of Molecular Sciences, 13*(12), 17210–17229.
59. Oh, S. M., Kim, H. R., Yong, J. P., Lee, S. Y., & Chung, K. H. (2011). Organic extracts of urban air pollution particulate matter ($PM_{2.5}$)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells). *Mutation Research, 723*(2), 142–151.