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Research Paper

DNA damage and repair, oxidative stress and metabolism biomarker responses in lungs of rats exposed to ambient atmospheric 1-nitropyrene

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ABSTRACT

1-Nitropyrene (1-NP) is a mutagenic and carcinogenic pollutant very widespread in the environment. However, the relative investigations on genotoxicity, oxidative stress and metabolic enzymes in lungs of mammalian caused by 1-NP have not been fully established. In this study, the 1-NP solutions at 3 dosages (1.0×10^{-5}, 4.0×10^{-5} and 1.6×10^{-4} mg/kg body weight) were respectively given to rats by the intratracheal instillation. The responses of 1-NP on DNA damage and repair, oxidative stress and metabolism biomarkers in rat lungs after exposure to 1-NP were measured. The results showed 1-NP at three dosages induced obvious DNA strand breaks, 8-OH-dG formation and DNA-protein cross-link in rat lungs compared with the control. Higher dosage 1-NP (4.0×10^{-5} and 1.6×10^{-4} mg/kg body weight) greatly activated DNA repair gene OGG1 and inhibited MTH1 and XRCC1 expressions, and they significantly elevated the levels of GADD153, heme oxygenase-1 and malondialdehyde and decreased SOD activity, accompanied by the increases of CYP450, CYP1A1, CYP1A2 and GST levels. These results suggested the genotoxicity of 1-NP might rely on 1-NP-caused DNA damage and its combined effects on the suppression of DNA repair and the enhancement of oxidative stress and metabolic enzyme activity.

1. Introduction

Nitro-polycyclic aromatic hydrocarbons (NPAHs) are widespread in the air environment mainly from two major sources: direct emissions (e.g., from coal combustion, diesel engines, cigarette smoke, cooked meat products and biomass burning), and secondary formation through atmospheric reactions of polycyclic aromatic hydrocarbons (PAHs) and nitrogen dioxide (Lin et al., 2015a). In particular, they were ubiquitously identified in the atmosphere and ambient fine particulate matter ($PM_{2.5}$) in America, Europe and Asia (Ringuet et al., 2012; Jariyasopit et al., 2014), with relatively higher levels of NPAHs in $PM_{2.5}$ were found in East Asia including China, Russia, Korea and Japan (Lin et al., 2015a, 2015b; Hayakawa, 2016). NPAHs arouse extensive concern primarily because they not only are wide-spread environmental pollutants, but also possess much higher direct-acting mutagenicity and carcinogenicity than the parent PAHs, especially related to the development of lung, skin and bladder cancer (Yang et al., 2010; Wang et al., 2011; IARC, 2014). 1-Nitropyrene (1-NP), a representative of NPAHs and the most abundant NPAH, has been detected in many environmental samples including air particulate, coal combustion fly ash, diesel engine exhaust, cigarette smoke (Albinet et al., 2007; Ding et al., 2012; IARC, 2013). It has been listed as an IARC Group 2A carcinogen (IARC, 2016), indicating it is possibly carcinogenic to humans and has potential respiratory health risks. From laboratory experimental data, 1-NP was found to be mutagenic in mutagenicity test using bacteria and mammalian cells (Varga and Szendi, 2006; Watt et al., 2007), and mammary tumors were induced in rats treated with 1-NP for 86 weeks (Imaida et al., 1995). 1-NP enhanced pro-inflammatory gene expression in cultured BEAS-2B cells and generated excessive 8-hydroxydeoxyguanosine (8-OH-dG) and reactive oxygen species (ROS) in A549 cells (Kim et al., 2005; Park and Park, 2009). Genotoxicity is refers to the property of chemical agents that may damage the genetic information (such as DNA or chromosome) within a cell, causing mutations (which may lead to cancer). However, to our best knowledge, studies on the genotoxicity roles of 1-NP are relatively shortage, therefore evaluating the genotoxicity of 1-NP and investigating the related mechanisms are vital to reveal the environmental health hazards of 1-NP.

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Lung is a target organ that encounters most environmental chemicals. Previous studies indicated that the genotoxicity of environmental pollutants was linked to oxidative stress and DNA damage caused by the chemicals (Risom et al., 2005; Hanot-Roy et al., 2016), and related to the upregulation of metabolic enzymes (Shah et al., 2016). Generally, phase I xenobiotic-metabolizing enzymes like in particular cytochromes P450 (CYP450) and phase II enzymes including glutathione S-transferase (GST) may undergo a process of biotransformation and catalysis, accompanied by the formation of metabolites or intermediates, in which some metabolic active intermediates may mediate oxidative and reactive with DNA highly (Huang and Hu, 2014). In some cases, intermediates like ROS are produced in the process of metabolic bioactivation mediated by CYP450, triggering oxidative stress and leading to DNA damage if it is not repaired before replication (Moller et al., 2014; Hrycay and Bandiera, 2015). Notably, DNA repair genes play important roles in DNA damage repair processes. If normal DNA repair processes fail, DNA damage may occur (Sugasawa, 2016). Taken together, lung genotoxicity induced by chemicals is comprehensive and complex, in which DNA damage, oxidative stress, and metabolic disturbance are interrelated. As a $PM_{2.5}$-bound NPAH component, 1-NP could be oxidized by human P450 2A13 to form mono- and di-oxygenated products (Shimada et al., 2016). It could generate ROS in A549 cells and induce DNA damage along with the slower rate of DNA repair (Kim et al., 2005; Andersson et al., 2009). However, the detailed mechanisms of DNA damage and repair, oxidative stress and metabolic activation in laboratory animals induced by 1-NP have not fully been investigated so far. Accordingly, in this study, we focused on the DNA damage markers like DNA strand breaks, 8-OH-dG and DNA-protein cross-link (DPC), and multiple DNA damage repair genes, such as 8-Oxoguanine DNA glycosylase (OGG1), MutT Homolog 1 (MTH1) and X-ray repair cross-complementing group 1 (XRCC1), which were have been demonstrated to play key roles in DNA repair processes and be involved in mammalian nucleotide excision repair (Thacker and Zdzienicka, 2003; Nakabeppu, 2001). Meanwhile, growth arrest- and DNA damage-inducible gene 153 (GADD153) can be highly promoted when DNA damage or oxidative stress is initiated by envionmental pollutants (Fontanier-Razzaq et al., 2001; Tang et al., 2002), while heme oxygenase 1 (HMOX-1), superoxide dismutase (SOD) and malonaldehyde (MDA) may be obviously induced when oxidative stress occurs in the cells under the oxide stimulus (Takahashi et al., 2004; Tsikas, 2016). So GADD153, HMOX-1, SOD and MDA were used as the inducible and typical factors to explore the oxidative stress in rat lungs induced by 1-NP. In addition, the changes of phase I enzymes CYP450s isoforms (CYP1A1 and 1A2) and phase II enzyme GST in rat lungs were investigated to indicate the lung metabolic characteristic of 1-NP. Our data will clarify the toxicological roles in DNA damage and repair, oxidative stress and metabolic activation induced by 1-NP in depth and will provide new insight into evaluation the genotoxicity of atmospheric 1-NP exposure.

2. Materials and methods

2.1. Animal and treatment protocols

Male Wistar rats (body weight 180–200 g) were obtained from Animal Center of Hebei Medical University (Shijiazhuang, China) and bred in an animal house in Institute of Environmental Science of Shanxi University (Taiyuan, China) under standard conditions (24 °C ± 2 °C and 50% ± 5% humidity). Animals received food and water ad labium, except during the exposure period. Rats were divided randomly into four equal groups with five animals for each group: (1) the control (5% dimethyl sulfoxide, DMSO), (2) low dose 1-NP group (1.0×10^{-5} mg/kg b.w. 1-NP in DMSO), (3) medium dose 1-NP group (4.0×10^{-5} mg/kg b.w. 1-NP in DMSO, and (4) high dose 1-NP group (1.6×10^{-4} mg/kg b.w. 1-NP in DMSO). The rats were administered using 5% DMSO and 1-NP solutions by intratracheal instillation respectively every other day for 10 days. The research revealed that no histopathological abnormalities were observed in lung tissues of the control mice after administration of 5% DMSO (Martínez-González et al., 2014). All animal procedures were approved by the Shanxi University Animal Investigational Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by Ministry of Health People's Republic of China.

In this study, the concentration of 3.04 ng/m^3 of 1-NP, which was detected in $PM_{2.5}$ sample in Taiyuan of China (Ma et al., 2015), was used to estimate the 1-NP instillation dosage for each rat every 2 days as about 1.0×10^{-5} mg/kg b.w. by taking the respiratory volume limit of an adult rat (200 mL/min, 200 g/rat) into account. Moreover, according to the orange alert criterion of haze $PM_{2.5}$ in China (500 μg/m^3) and the approximate proportion of mass concentration of NPAHs in wintertime $PM_{2.5}$ ranging from 9×10^{-6} to 1×10^{-4} (Lin et al., 2015a), 4.0×10^{-5} mg/kg b.w. of 1-NP dosage was used when 2.7×10^{-5} of 1-NP proportions in $PM_{2.5}$ were chosen. Based on above considerations and requirement of dose-response relationship experiment design, three concentrations of 1-NP were selected to be 1.0×10^{-5}, 4.0×10^{-5} and 1.6×10^{-4} mg/kg b.w. in this study.

After the last treatment, rats in different groups were euthanized and sacrificed, and then a piece of fresh lung tissue per rat was minced and grinded for comet assay, another part was homogenized for ELISA and biochemical analysis, and the rest lung tissue was quickly frozen in liquid nitrogen and then stored at −80 °C for mRNA and protein measurement.

2.2. Comet assay

The alkaline comet assay was performed as follows. (1) Single cell suspensions were prepared in ice-cold phosphate-buffered saline (PBS) from a piece of lung (See "2.1" Section). (2) Preparation of "sandwich gel". First layer was 1% normal melting-point agarose (NMA) in a slide; second layer was the mixture of cell/0.65% molten low melting-point agarose (LMA), third layer was 0.65% LMA. (3) The slides containing "sandwich gel" were transferred to cold lysis solution (2.5 mM NaCl, 100 mM EDTA, 1% sodium sarcosinate and 10 mM Tris, pH 10.0, to which 1% Triton X-100 and 10% DMSO were freshly added) for 60 min at 4 °C to cause denaturation. (4) The slides were then subjected to electrophoresis with cold electrophoreses buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) at 25 V for 30 min at 4 °C, and immersed in Tris buffer (0.4 M Tris, pH 7.5) to neutralize the excess alkali. Subsequently the slides were air dried. (5) DNA was stained with 100 μL 4S Red Plus (Shengon, Shanghai, China, 1:10,000) for 20 min and immediately rinsed with Milli-Q water and air dried. (6) Slides were examined at 400× magnification using an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan). 20–30 randomly acquired images of microscopic fields compared to each sample were recorded to enable analysis of 100–150 cells. (7) DNA damage indexes including comet tail DNA%, tail length, and olive tail moment (OTM) were assessed by a Comet Assay Software Project (CASP, CASP, version 1.2.3 beta1).

2.3. Real time quantitative RT-PCR

Lung tissues (See "2.1" Section) in different group rats were used to mRNA extraction and quantitative RT-PCR analysis of tested genes and housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was referenced as described previously (Li et al., 2015). Expression levels were assessed by real-time PCR in an iCycler iQ Real Time PCR Detection System (Bio-Rad, Richmond, CA, USA) with the Quantitect SYBRGreen I PCR kit. The GenBank accession numbers and the primer sequences of the tested genes with the PCR product amplified fragments and annealing temperature are listed in Table 1. The relative quantification of the expression of the target genes was measured using GAPDH mRNA as an internal control. The copy numbers of target gene/GAPDH mRNA ratio were measured in all samples.