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$PM_{2.5}$ exposure during pregnancy induces hypermethylation of estrogen receptor promoter region in rat uterus and declines offspring birth weights☆

Shaokang Dang [a], Ding Ding [a], Yong Lu [b], Qian Su [a], Tianwei Lin [c], Xiaoxiao Zhang [c], Huiping Zhang [a], Xuebin Wang [d], Houzhang Tan [d], Zhongliang Zhu [c], Hui Li [a, *]

[a] Division of Neonatology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China
[b] Department of Genetics, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, 710061, China
[c] Key Laboratory of Shaanxi Province Biomedicine, College of Life Sciences, Northwest University, Xi'an, Shaanxi, 710061, China
[d] Department of Thermal Engineering, Energy and Power Engineering College of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China

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ABSTRACT

Particulate matter 2.5 ($PM_{2.5}$) exposures during pregnancy could lead to declined birth weight, intrauterine developmental restriction, and premature delivery, however, the underlying mechanisms are still not elucidated. There are few studies concerning the effects of $PM_{2.5}$ exposure on maternal and child health in Xi'an (one of the cities with severe air pollution of $PM_{2.5}$ in North China). Then, this study aimed to investigate the effect of $PM_{2.5}$ exposure in Xi'an on the offspring birth weights and the possibly associated epigenetic mechanisms. We found the Low and High groups: the offspring with declined birth weights; the decreased mRNA and protein expression of the estrogen receptor (ERs) and eNOs in the uterus; the decreased endometria vascular diameter maximum (EVDM); the increased mRNA and protein expressions of the DNMT1 and 3b in the uterus; the elevated methylation levels of the CpG sites in the CpG island of ERα promoter region in the uterus. However, no differences were observed in the mRNA or protein expressions of ERβ and DNMT3a between the Clean and $PM_{2.5}$ exposure groups, as well as endometriavascular density (EVD). Additionally, $PM_{2.5}$ level was negatively correlated with the ERα protein expression, EVDM and offspring birth weight, as well as the methylation level of the CpG sites in the CpG island of ERα promoter region and the ERα protein expression in the uterus; whereas the ERα protein expression was positively correlated with the offspring birth weight, as well as $PM_{2.5}$ level and the methylation level of the CpG sites in the CpG island of ERα promoter region in the uterus. Taken together, elevated methylation level of the CpG sites in the CpG island of ERα promoter region reduces ERα expression in the uterus, which could be one of the epigenetic mechanisms that pregnant $PM_{2.5}$ exposure reduces the offspring birth weights.

1. Introduction

Over the past decade, more and more investigation has shown that particulate matter 2.5 ($PM_{2.5}$) exposure during pregnancy could lead to declined birth weight, intrauterine developmental restriction, and premature delivery (Dadvand et al., 2013; Hjortebjerg et al., 2016; Trasande et al., 2016), even though the underlying mechanisms are still needed to be fully established. Considering that $PM_{2.5}$ pollution around world had spatial and temporal distribution specificity, at present, there are few studies concerning the effects of $PM_{2.5}$ exposure on maternal and child health in Xi'an (one of the cities with severe air pollution of $PM_{2.5}$ in North China). PM refers to the mixture of solid and liquid mixtures consisting of motor vehicle exhaust, road dust, power plant dust, and eolian dust, especially the fine PM suspended in the air (with the gas dynamics diameter of $\leq$2.5 μm, i.e., $PM_{2.5}$), which has been shown to be closely associated with the pathogenesis of cardiovascular diseases (Xu et al., 2011). Previous clinical investigation has shown that exposure of $PM_{2.5}$ during pregnancy might lead to

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* Corresponding author. Division of Neonatology, the First Affiliated Hospital of Xi'an Jiaotong University, No. 277, YantaWest Road, Xi'an, Shanxi, 710061, China.
E-mail address: huili@mail.xjtu.edu.cn (H. Li).

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trophoblast invasion abnormalities and high vascular resistance via impairing the uterine spiral artery modification and arterial adaptive angiogenesis, and, uterine placental interface blood flow would be reduced, which negatively affects the fetal oxygen and nutrition supply, then resulting in series of negative outcomes in offspring (van den Hooven et al., 2012).

During pregnancy, the uterine blood flow (UBF) would significantly increase by as much as 50 times, providing adequate nutrition and oxygen to support the placental function and fetal growth (Rosenfeld, 1977). Studies have shown that the vascular adaptive alterations in the uterus during pregnancy are largely mediated by the ERs-induced eNOs expression up-regulation (Magness et al., 2001; Rupnow et al., 2001; Vagnoni et al., 1998) and activity enhancement (Liao et al., 2005; Magness et al., 1996; Salhab et al., 2000; Yi et al., 2010), as well as the eventually increased NO production (Miller et al., 1999; Rosenfeld et al., 1996). In addition, during pregnancy, ERs-mediated uterine angiogenesis might be another mechanism for the increased UBF (Magness and Rosenfeld, 1989; Miller et al., 1999). Therefore, ERs have important physiological effects on uterine vascular function, especially during pregnancy. Inversely, ERs abnormalities during pregnancy would result in insufficient UBF, as well as nutrition and oxygen transportation shortages, further leading to restricted fetal intrauterine growth and increased neonatal morbidity and even mortality (Lang et al., 2003). In ovariectomized sheep models, treatment of non-specific ERs antagonists could inhibit the exogenous estrogen-induced UBF elevation (up to 70%) (Magness et al., 2005). Moreover, compared with wilt-type or ERβ knockout mice, the NO-mediated vasodilatation would be significantly attenuated in the ERα knockout mice (Dupont et al., 2000; Rubanyi et al., 1997); while compared with the wild-type or ERα knockout mice, the proliferation and migration of vascular endothelial cells would be severely impaired in the ERβ knockout mice (Wang et al., 2003). Together, these findings suggest that ERs are important molecular target in the clinical research of pregnancy-related complications.

Previous studies have reported that epigenetic regulation of gene transcription (including post-translational histone modification and DNA methylation), is a process particularly sensitive to environmental pollution, which might even remain lifelong (Baillie et al., 2011; Belinsky et al., 2002; Byun et al., 2013; Janssen et al., 2013; Ji et al., 2016; Kingsley et al., 2016; Reik, 2007). Furthermore, the exposure to environmental pollution during pregnancy has been shown to have a significant impact on the epigenetic regulation of ERs gene transcription (Li et al., 2003). For example, in mice, treatment of bisphenol A (a substance attached to the surface of airborne fine particles) at the dose equivalent to the environment of during gestation could induce methylation modifications changes in the promoter of ERs gene in hippocampal tissues of female offspring (Kundakovic et al., 2013). Another study in Belgium has suggested that the declined methylation level in placenta of pregnant women is closely related to the $PM_{2.5}$ exposure (Janssen et al., 2013). Based on these findings, in this study, the effects of $PM_{2.5}$ exposure on the methylation modification in uterus ERs promoter region were investigated in rat models.

2. Materials and methods

2.1. Preparation of $PM_{2.5}$

Firstly, the averaged daily concentration of the $PM_{2.5}$ exposure on A [SPF (specific pathogen free, SPF) animal room, College of Life Sciences, Northwest University, Xi'an, Shaanxi, China], B and C (respectively, in the indoor of the long- and short-distance from Boiler Workshop, Heating Company, Xi'an, Shaanxi, Chan) were assessed with the Dekati® Low Pressure Impactor (DLPI; Dekati Ltd., Tampere, Finland), DLPI collected $PM_{2.5}$ at a speed of 9.93 L/min into filter (diameter<2.5 μm). Briefly, the every consecutive 24 h monitoring of the $PM_{2.5}$ exposure in indoor A, B, and C was random performed, respectively, during 21 days (from 1 to 21, January 2016), and the total number of random monitoring times in indoor A, B, and C was obtained respectively on 10, 12, and 8 days. The averaged daily concentration of the $PM_{2.5}$ exposure on 10, 12 and 8 days were then respectively averaged. Moreover, the averaged daily $PM_{2.5}$ value over 21 consecutive days (from 1 to 21, January 2016) from the Monitoring Station, Environmental Protection Agency, Xi'an, Shaanxi, China was also obtained (Table 1). Based on these data, the exposure averaged daily concentrations of $PM_{2.5}$ on the pregnant rats were determined to mimic the character of the averaged daily concentration in Xi'an outdoor. Accordingly, the averaged daily $PM_{2.5}$ concentration >150μg/m^3, between 75 and 150 μg/m^3 (75μg/m^3< $PM_{2.5}$ ≤150μg/m^3), ≤75μg/m^{3}was designated respectively as the high-, low-exposure and clean group (indoor C, B and A).

2.2. Study animals and tissue preparation

Sprague-Dawley rats were provided by the Animal Research Center of the School of Medicine, Xi'an Jiaotong University (Xi'an, Shaanxi, China). They were selected because of large litter size, rapid growth and development, and strong resistance to disease (especially strong resistance to respiratory diseases), which were suitable for the toxicological experiments for air pollution (Pinnamaneni et al., 2014; Zeng et al., 2018). These animals were housed in a 12 h: 12 h day-night cycle, at the temperature of 22 °C in the humidity of 60%, with free access to food and water. Totally 18 female rats (weighing 230–250 g, 9-week old) and 6 male rats (weighing 210–250 g, 9-week old) were first housed separately for adaptive feeding. Then, from 20:00 to 22:00 every night, the male and female rats were put into one cage at the rate of 3:1 to be allowed to mate. On the next morning, the female rats were subjected to the vaginal smear observation, and the female animals with sperms covering the full-field of view were recognized as pregnancy (at day 0). The pregnant rats were housed separately on days 0–21 of pregnancy, which were then randomly divided into the clean (Clean), low (Low), and high (High) groups (n = 6 each group). The pregnant rats from Clean, Low and High groups were respectively exposure into indoor A, B and C for consecutive21 days. On the first day after birth, 1 and/or 2 rats was random picked out in every litter from every group, the picked total 6, 9, 8 rats from corresponding Clean, Low and High group (both male and female) were respectively weighted by an AL model electronic balance (Mettler-Toledo Co., Ltd., Columbus, OH, USA). Immediately after delivery, the maternal rats were anesthetized with chloral hydrate injection and subjected to decapitation. The uterine tissues were rapidly removed and stored at −80 °C. All animal experiments were conducted according to the ethical guidelines of Medical School of Xi'an Jiaotong University. The experimental protocol was approved by Medical School of Xi'an Jiaotong University.

2.3. Quantitative real-time PCR

Total RNA was extracted from the uterine tissue with the RNA-fast200 kit (Fastagen, Shanghai, China). The cDNA was synthesized with the reverse transcription kit (PrimeScript ™ RT Master Mix, Code No. RR036A; TAKARA, Kyoto, Japan). Real-time PCR was performed with the SYBR® Fast qPCR Mix Fast kit (Code No. RR430A; TAKARA), according to the manufacturer's instructions. Primer sequences were shown in Table 2 (synthesized by Sangon Biotech, Shanghai, China). Quantitative real-time PCR was performed according to the previously published protocols (Yoshizaki et al.,