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Gestational 1-nitropyrene exposure causes fetal growth restriction through disturbing placental vascularity and proliferation

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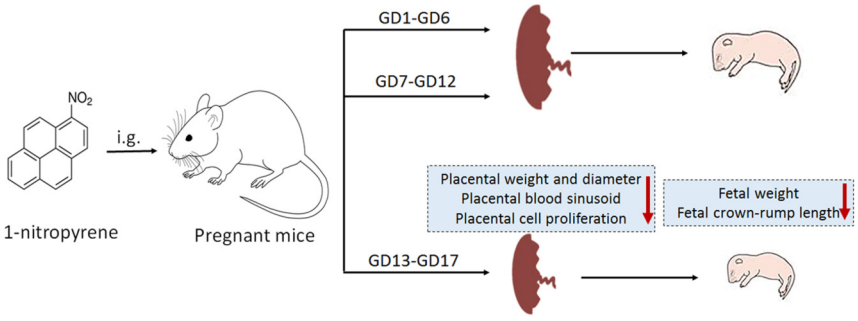
HIGHLIGHTS

- Gestational 1-NP exposure reduces fetal weight in a stage-dependent manner.
- Gestational 1-NP exposure reduces crown-rump length in a stage-dependent manner.
- Maternal 1-NP exposure in late pregnancy causes blood sinusoid reduction.
- Maternal 1-NP exposure in late pregnancy inhibits placental cell proliferation.

GRAPHICAL ABSTRACT



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ABSTRACT

1-Nitropyrene (1-NP) is a widely distributed pollutant in the environment and is best known for its mutagenicity and carcinogenicity. In this study, we evaluated the effects of 1-NP exposure in different gestational stages on the pregnant outcomes. Pregnant mice were administered with 1-NP by gavage daily in early (GD1-GD6), middle (GD7-GD12) or late pregnancy (GD13-GD17), respectively. We found that gestational 1-NP exposure had no effect on implantation sites per litter, preterm delivery and fetal death. Interestingly, mice exposed to 1-NP in late pregnancy showed a significant reduction in fetal weight and crown-rump length. Correspondingly, placental weight and diameter were markedly reduced in dams exposed to 1-NP in late pregnancy. Additional experiment showed maternal 1-NP exposure in late pregnancy reduced blood sinusoid area of placental labyrinthine region in a dose-dependent manner. Although gestational 1-NP exposure had little effect on placental cell apoptosis, as determined by the TUNEL assay, the rate of Ki67-positive cell, a marker of cell proliferation, was reduced in placental labyrinthine region of mice exposed to 1-NP in late pregnancy. These findings provide evidence that gestational 1-NP exposure induces fetal growth restriction in a stage-dependent manner. Placenta is a toxic target in the process of 1-NP-induced fetal growth restriction.

1. Introduction

1-Nitropyrene (1-NP), with one nitro-functional group on the benzene ring of pyrene, is a representative nitrated polycyclic

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aromatic hydrocarbon (nitro-PAH) in the environment (Bandowe and Meusel, 2017). As a by-product of combustion, a high level of 1-NP has been detected in diesel exhaust particle (DEP), coal fly ash and urban air particulate (Harris et al., 1984; Bamford et al., 2003; Lin et al., 2015; Wang et al., 2016a,b). In addition, 1-NP has also been detected in sediment (Huang et al., 2014), river water (Ohe and Nukaya, 1996) and food (Yaffe et al., 2001; Deng and Chan, 2017). Human is generally exposed to 1-NP through both inhalation and ingestion, while food ingestion is the predominant pathway as previously reported (Yaffe et al., 2001; Stenerson et al., 2015; Deng and Chan., 2017). 1-NP, well-known for its mutagenicity and carcinogenicity (Edwards et al., 1986; Imaida et al., 1995; Chatel et al., 2014), has been identified as Group 2A carcinogen by the International Agency for Research on Cancer (IARC) in 2012 (IARC, 2012). Recently, researchers have begun to explore the adverse effects of 1-NP beyond mutagenicity and carcinogenicity (Park and Park, 2009; Podechard et al., 2011; Onduka et al., 2017). However, little datum exists for 1-NP with developmental toxicity endpoints.

Intrauterine growth restriction (IUGR) or fetal growth restriction (FGR) refers to the inability of a fetus to achieve his or her genetically determined potential size (Resnik, 2002). Generally, the estimated fetal weight (EFW) and/or abdominal circumference (AC) less than the 3rd centile can be diagnosed as IUGR in clinic (Gordijn et al., 2016). As the major adverse effect observed in developmental toxicity studies, IUGR can not only increase the risk of infant morbidity but has also been associated with metabolic diseases and mental disorders in adulthood (Victora et al., 2008; Crume et al., 2014). IUGR is a result of various etiologies, including maternal, placental and fetal factors (Sharma et al., 2016). In recent decades, the correlation between gestational exposure to environmental pollutants and the incidence of IUGR has been validated by a large number of epidemiologic studies. (Choi et al., 2008; Wesselink et al., 2014; Cao et al., 2016; Wang et al., 2016a,b, 2017; Geer et al., 2017). The underlying mechanism was likely due to the disruption of placental development and impairment of placental function after gestational pollutant exposure (Valentino et al., 2016; Zhang et al., 2016). As a temporary but highly specialized organ, placenta plays vital roles in nutrients and waste products transport, immunomodulation and endocrine regulation (Gude et al., 2004). Several reports from rodent animals indicate that some pollutants, such as benzo[*a*]pyrene and di (2-ethylhexyl) phthalate, could induce IUGR through damaging placental development (Sanyal and Li, 2007; Shen et al., 2017).

The development toxicity of a certain pollutants is largely on account of exposure period. In this study, we aimed to investigate whether gestational 1-NP exposure induces fetal IUGR in a stage-dependent manner in a mouse model. In addition, placental development was also evaluated to explore whether placenta is a toxic target in the process of 1-NP-triggered adverse pregnancy outcomes. This study could be valuable for revealing the etiology of IUGR from the view point of pollutants-induced placental impairment.

2. Materials and methods

2.1. Animals and treatment

8-week-old male and female CD-1® (ICR) mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Mice were maintained at controlled temperature (20–25 °C) and humidity (50 ± 5%) environment on a 12-h light/12-h dark cycle with free access to food and water. The protocols and the use of animals were approved by the Anhui Medical University Animal Care and Use Committee. Pregnant mice were obtained as previously described (Shen et al., 2017). Briefly, female mice were housed overnight with male mice starting at 21:00 p.m. Females were checked at 7:00 a.m. the next morning, and the presence of a vaginal plug was identified as gestational day (GD) 0. Pregnant mice were randomized into six groups: early-1-NP, early-control, middle-1-NP, middle-control, late-1-NP and late-control. Pregnant mice in early-1-NP, middle-1-NP and late -1-NP groups were administered with 1-NP (100 μg kg^{-1} body weight, dissolved in corn oil) by gavage daily in early pregnancy (GD1-GD6), middle pregnancy (GD7-GD12) or late pregnancy (GD13-GD17), respectively. Accordingly, pregnant mice in early-control, middle-control and late-control groups received corn oil by gavage daily on GD1-GD6, GD7-GD12 and GD13-GD17, respectively. On GD18, all mice were sacrificed. The number of live fetuses, dead fetuses and implantation sites were counted for each litter of mice. And for live fetuses, body weight, crown-rump length, placenta weight and placenta diameter were measured. Besides, placental histopathology and immunohistochemistry assay were performed with pregnant mice gavaged with different doses of 1-NP (1, 10 or 100 μg kg^{-1} body weight) daily in the target stage that was proved by the above experiments.

2.2. Placental histopathology

Fresh placenta tissues were treated as previously described (Shen et al., 2017). Briefly, placentas were fixed in 4% paraformaldehyde and then embedded in paraffin. After cutting, tissues were deparafnized and stained with hematoxylin and eosin (H&E). The areas of blood sinusoids in labyrinthine region were analyzed in placental sections. Five fields were selected randomly in the labyrinthine region of each section at magnification ×400. The average percentage of blood sinusoid areas was calculated as the ratio of the number of pixels covered by the area defined by the threshold to the overall number of pixels in the image. The Image J Program from NIH was applied to perform the image analysis.

2.3. Immunohistochemistry

Immunohistochemistry was performed on deparaffinized and rehydrated placental sections. After antigen retrieval and endogenous peroxidase blocking, placental sections were incubated with primary antibodies against Ki67 (ab16667, Abcam, Cambridge, UK) at 37 °C for 1 h. The color reaction was developed with HRP-linked polymer detection system and counterstaining with hematoxylin.

2.4. Terminal dUTP nick-end labeling (TUNEL) assay

The TUNEL staining was performed with the TUNEL kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, deparaffinized and rehydrated placental sections were fixed with 4% paraformaldehyde solution and then permeabilized with Proteinase K. After another fixation process, the sections were labeled by adding rTdT reaction mix. The reaction was stopped after incubation at 37 °C for 1 h in a humidified chamber. Then the 0.3% hydrogen peroxide was applied to block endogenous peroxidase of placental sections. And then the sections were incubated with treptavidin-HRP solution followed by chromogenic substrate 3,3 -diaminobenzidine (DAB) treatment. Lastly, the sections were counterstained with hematoxylin.

2.5. Statistical analysis

Quantified data were presented as mean ± standard error of the mean (S.E.M.). The litter was considered as the unit for statistical comparison among different groups. Student's t-test, chi-square test, repeated measures ANOVA or One-Way ANOVA followed by