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Exposure to benzo[*a*]pyrene impairs decidualization and decidual angiogenesis in mice during early pregnancy☆

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ABSTRACT

Benzo[*a*]pyrene (BaP) is a ubiquitous environmental persistent organic pollutant and a well-known endocrine disruptor. BaP exposure could alter the steroid balance in females. Endometrium decidualization and decidual angiogenesis are critical events for embryo implantation and pregnancy maintenance during early pregnancy and are modulated by steroids. However, the effect of BaP on decidualization is not clear. This study aimed to explore the effects of BaP on decidualization and decidual angiogenesis in pregnant mice. The result showed that the uteri in the BaP-treated groups were smaller and exhibited an uneven size compared with those in the control group. Artificial decidualization was detected in the uteri of the controls, but weakened decidualization response was observed in the BaP-treated groups. BaP significantly reduced the levels of estradiol, progesterone, and their cognate receptors ER and PR, respectively. The expression of several decidualization-related factors, including FOXO1, HoxA10, and BMP2, were altered after BaP treatment. BaP reduced the expression of cluster designation 34 (CD34), which indicated that the decidual angiogenesis was inhibited by BaP treatment. In addition, BaP induced the downregulation of vascular endothelial growth factor A. These data suggest that oral BaP ingestion compromised decidualization and decidual angiogenesis. Our results provide experimental data for the maternal reproductive toxicity of BaP during early pregnancy, which is very important for a comprehensive risk assessment of BaP on human reproductive health.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants that are widely released in the environment (Yu et al., 2014). Recently, PAH pollution has become a global concern. Enriched concentrations of PAHs are found in the street dust of major West African metropolises, indicating the influence of traffic emission (Bandowe and Nkansah, 2016). The concentrations of PAHs in work places and sediments have also been studied in different countries (Campo et al., 2012; Dudhagara et al., 2016; Evans et al., 2016). In China, the environmental distribution of PAHs is well reported. Many studies have investigated the contamination of PAHs in street dust and in surface sediments of different river estuaries, sea, and sea bays (He et al., 2014; Li et al., 2010b; Qian et al., 2016; Song et al., 2015; Zhang et al., 2016). In some special areas such as the coal district of South China, heavy contamination with PAHs are found in the soil and water, posing a significant health risk to residents, particularly coal workers (Huang et al., 2016). Benzo[*a*]pyrene (BaP) is a representative compound among PAHs. The main sources of BaP in the environment include volcanic eruptions, incomplete burning of coal and wood, automobile exhausts, and smoking. People could be exposed to BaP through the inhalation of polluted air and ingestion of contaminated water and food, which may cause an accumulation of BaP in the body and result in adverse effects (Archibong et al., 2002; Lu and Zhu, 2007). Currently, the carcinogenicity of BaP and its mechanisms are well established (Caiment et al., 2015). In recent years, the effects of BaP on reproductive health have received increased attention. Studies

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on the reproductive toxicity of BaP are primarily focused on its effects on sex hormones, embryogenesis, and fetal survival (Kummer et al., 2013; Machado Jde et al., 2014; Ramesh et al., 2001). Females who are exposed to BaP could exhibit ovarian impairment and developmental delays (Miller et al., 1992). Fetal exposure to BaP could affect survival and luteotropic activity in the exposed animals (Archibong et al., 2002). Postnatal exposure to BaP altered ovarian estrogen receptor (ER) expression, impaired ovarian morphology, and disturbed ovarian function in immature rats (Kummer et al., 2013). However, these studies rarely focused on the effect of BaP exposure in early pregnancy.

Decidualization is a crucial event in early pregnancy. After implantation, the endometrial stromal cells begin to undergo decidualization for subsequent embryo development and placentation. Proper physiological and morphological changes in the maternal uterus are essential for successful decidualization. Improper decidualization can trigger pathological changes and lead to adverse pregnancy outcomes (Archibong et al., 2002). The steroid hormones estradiol (E2) and progesterone (P4) regulate decidualization in coordination with their cognate receptors, the ER and P4 receptor (PR), respectively (Ramathal et al., 2010). The decidual/trophoblastic PRL-related protein (d/tPRP), a well-characterized biochemical marker, is induced during this process. Homeobox A10 (HoxA10), bone morphogenetic protein-2 (BMP2), and Indian hedgehog (IHH) have been confirmed as targets of E2 and P4 during decidualization. Many studies have revealed that forkhead box O1 (FOXO1), cyclooxygenase-2 (COX2), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) are key regulators of successful decidualization. BaP was reported to be an endocrine disruptor, which can affect the secretion of P4 and E2 (Archibong et al., 2002). Nevertheless, the influence of BaP on decidualization remains unknown.

In early pregnancy, decidua formation is accompanied by abundant neovascularization and vascular remodeling, which permit the uterus and the embryo to acquire adequate oxygen and nutrients before placentation (Cha et al., 2012; Dey et al., 2004; Ramathal et al., 2010). It has been reported that vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) are very crucial for angiogenesis and are modulated by steroid hormones and other factors (Plaisier, 2011; Smith, 2000; Zygmunt et al., 2002). BaP has been proved to be vasculotoxic and can inhibit neovascularization and angiogenesis in mice and human umbilical vein endothelial cells (Ichihara et al., 2009; Li et al., 2010a). Comparable reports on BaP in decidual angiogenesis during early pregnancy are scarce.

Although several studies have investigated the effect of BaP on reproductive health, most of them are focused on pregnancy outcomes and postnatal development (Archibong et al., 2012; Kummer et al., 2013). Because maternal uterine decidualization is also very important for pregnancy maintenance and embryo development, the present study aimed to explore the effects of BaP on maternal uterine decidualization and decidual angiogenesis in mice during early pregnancy. Our research may provide a new direction to illustrate the reproductive toxicity of BaP.

2. Materials and methods

2.1. Ethical approval

Animal experiments were authorized by the Ethics Committee of Chongqing Medical University. All surgeries and sacrifice of animals were accomplished under anesthesia, and best efforts were made to lessen animal suffering.

2.2. Chemicals

Corn oil and BaP (96% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Animals and treatments

Kunming mice (6–8 weeks old, weighing 25–30 g) were supplied by the Animal Facility of Chongqing Medical University, China (Certificate No.: SCXK (YU) 20070001). The animals were housed at 22 ± 2 °C and light-controlled (12-h light/12-h dark cycle) conditions with free access to water and food. Polypropylene cages and glass water bottles were used to avoid unintentional exposure to other endocrine-active compounds such as bisphenol-A. Adult female Kunming mice were mated with fertile or vasectomized Kunming males to produce pregnancy or pseudopregnancy, respectively. The presence of a vaginal copulation plug was considered to indicate day 1 of pregnancy or pseudopregnancy. Pregnant mice were numbered with picric acid staining and then randomly divided into a control group and three treatment groups using a complete randomization procedure. The mice in the control group were gavaged with corn oil. In the treatment groups, the mice were administered three concentrations of BaP (0.2, 2, and 20 mg/kg body weight). BaP was dissolved in corn oil and administered daily by oral gavage at 0.05 mL/10 g of body weight from day 1 to day 8. During the exposure period, all mice were observed for toxic effect. Body weight was recorded every day. No mice died in the experimental process. Pregnant mice were decapitated in the morning on days 6–8, and the uteri were collected after morphological observations. The method of artificial decidualization was conducted according to previous research (Geng et al., 2015). To minimize subjective errors, morphological observations were performed twice by two persons who were blinded to the group assignment of the animals.

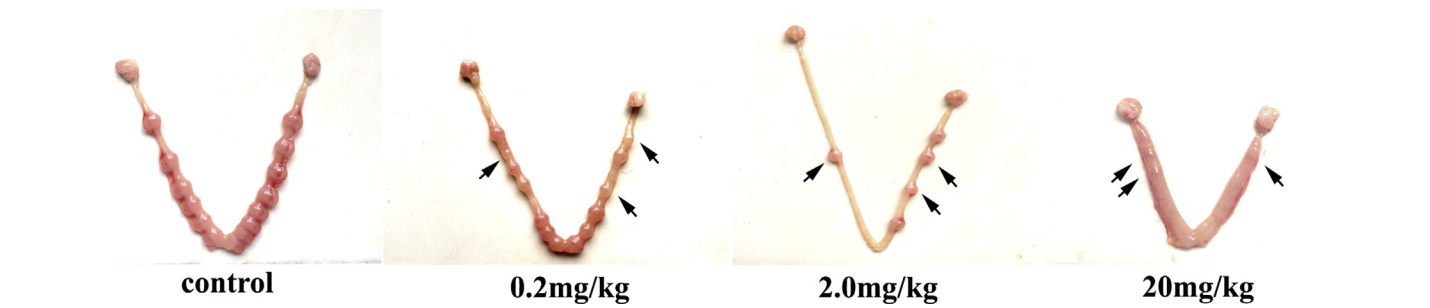

Fig. 1. BaP-treated mice exhibited abnormally sized implantation sites. The images show the morphology of the mouse uterus in the control and treated groups on day 7 of gestation. Arrows indicate abnormal implantation sites that are smaller than those of the control group. $n = 19$ in each group.

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