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Peroxisome proliferator activated receptor gamma in human placenta may mediate the adverse effects of phthalates exposure in pregnancy

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ABSTRACT

Peroxisome-proliferator activated receptor gamma (PPARG) in placenta play an important role in pregnancy. Our previous study showed that it mediated the effects of phthalates on placental mRNA expression of estrogen synthetases in rats. To assess the effects of phthalate exposure on PPARG placental expression, and the contribution of PPARG to the effects of phthalates in human. 207 healthy pregnant women were recruited and their cord blood and placenta were collected upon delivery. Three phthalates, estrogens in cord blood and protein expression of PPARG in placenta were measured. Linear regression were used to analyze the relationship between phthalates exposure, PPARG expression and hormones. Phthalate levels in cord blood were positively associated with PPARG protein expression in placenta ($p < 0.05$), whereas estrogens in cord blood were negatively associated with phthalate levels and PPARG expression ($p < 0.05$). This study shows that PPARG in placenta may mediate the adverse effects of phthalates on pregnancy in human.

1. Introduction

Phthalates, a class of synthetic chemicals, are commonly found in the environment because of its worldwide widespread use [1,2]. As we have previously reported, patients can be exposed to these compounds even in the hospital setting through intravenous infusions among other means [3]. Some of these compounds cross the placental barrier and can be detected in cord blood [4,5]. Recently, we found an association between phthalate levels in cord blood and preterm delivery and fetal growth parameters in Chinese women [3]. These associations were also confirmed by other studies [6,7], and suggest that exposure to phthalates in pregnant women may become a serious public health problem worldwide. However, the implications and mechanisms mediating these findings are incompletely understood.

Placenta is an endocrine gland that synthesizes peptides and steroid hormones during pregnancy including progesterone (P) and estrogens (estriol (E3), estradiol (E2)), which are essential for the maintenance of pregnancy. Xu's study indicates phthalates and their metabolites can inhibit the activity of 3-beta-hydroxysteroid dehydrogenase 1 (HSD3B1), a key catalyzing enzyme in both progesterone and estrogens biosynthesis in placenta, and cytochrome P450 family 19 subfamily A member 1 (CYP191a1), a key regulator of estrogens synthesis in placenta; thereby decreasing progesterone and estradiol levels [8]. In our previous study in rats, we showed that DEHP exposure can increase mRNA expression of four estrogen synthetases (17-beta-hydroxysteroid dehydrogenase 3, 4 and 7, (HSD17B3, HSD17B4 and HSD17B7) and HSD3B1), and

Abbreviations: PPARG, Peroxisome proliferator activated receptors gamma; E3, estriol; E2, estradiol; P, progesterone; MMPs, matrix metalloproteinase; DEHP, di-(2-ethylhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; DBP, Dibutyl phthalate; DIBP, Di-isobutyl phthalate; IV, intravenous infusion; BMI, body mass index; PROM, premature rupture of membranes; ICP, intrahepatic cholestasis of pregnancy; PIH, pregnancy induced hypertension syndrome (including preeclampsia); GDM, gestational diabetes mellitus; HC, head circumference; BPD, biparietal diameter; AC, abdominal circumference; FL, femur length.

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two inflammation-associated enzymes (matrix metalloproteinase 2 and 9 (MMP2 and MMP9)), and decrease mRNA expression of CYP191a1. It has been postulated that inhibiting the activity of peroxisome proliferator activated receptors gamma (PPARG) may redress these effects [9].

PPARG is a member of the super family of nuclear hormone receptors expressed in several tissues including placenta [10,11]. It binds to the target DNA along the retinoid X receptors and activates the expression of downstream genes which are associated with nutritional supply, hormonal secretion and inflammation [12–14]. It is important in pregnancy as it is thought to mediate preterm delivery and poor fetal growth [10,11,15–17]. Phthalates and their metabolites are peroxisome proliferators and could conjugated to PPARs (including PPARG) and activate them [18–21]. Recently, some studies found Dibutyl phthalate (DBP), one of the most commonly used phthalates, can not only activate PPARs but also increase their protein expression in Sertoli cells [21]. Our previous study demonstrated that di-(2-ethylhexyl) phthalate (DEHP), one of the most commonly used phthalate, can increase the protein expression of PPARG in ovaries and placenta in rats [22].

Based on this, we hypothesized that the adverse effects of phthalates on pregnancy outcomes may involve upregulation of PPARG expression in placenta, through which phthalates will modulate the secretion of hormones and inflammation-related proteins [23,24]. In this study we investigated the relationship between phthalate levels in cord blood with PPARG expression in placenta, hormone levels (E3, E2 and P), and inflammatory mediator expression (matrix metalloproteinase (MMPs)) in cord blood. We also sought to establish the association between PPARG expression, hormones and inflammatory mediator levels with preterm delivery and birth outcomes.

2. Material and methods

2.1. Study population

Characteristics including maternal age, prenatal examination, pregnancy history, pregnancy- associated diseases, gestational age, infant sex and birth outcomes from this cohort of 207 subjects have been previously reported [3]. Briefly, 207 eighteen to thirty-five years old, married, Han Chinese women, who delivered between October 2011 and September 2012 and had resided in Chongqing (Southwest China) for at least two years before delivery were recruited into this study. All subjects underwent prenatal examination and delivered at the department of gynecology and obstetrics, Southwest Hospital, Chongqing, China. Subjects were excluded if they had a history of alcohol or tobacco use, or family or personal history of occupational exposure to phthalates, or a history of disease which might affect pregnancy (hypertension, diabetes, psychosis, or other mental illnesses). Gestational age was determined by the last menstrual period and 33 of these 207 had preterm delivery (defined as a delivery with gestational age less than 37 weeks).

A questionnaire was administered to participants after labor to obtain information on socio-demographic characteristics (age, race, marital status, education, family or personal history of occupational exposure to phthalates), medical history (including previous pregnancy and obstetric history) and lifestyle risk factors (history of alcohol or tobacco use, negative life events (domestic violence, separation, long-term liabilities, unemployment)). Delivery characteristics and fetal growth parameters were obtained from the perinatal database at the Southwest Hospital in Chongqing where the patients delivered. This included maternal weight (before pregnancy and at delivery), maternal height, maternal heart rate and blood pressure at delivery, presence of pregnancy complications (including premature rupture of membranes (PROM), intrahepatic cholestasis of pregnancy (ICP), pregnancy induced hypertension syndrome (PIH, including preeclampsia), gestational diabetes mellitus (GDM)), intravenous infusion (IV) history (within 1 month before delivery), gestational age, birth weight, birth length, head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL).

Ten milliliters of cord blood were obtained from each subject within 10 min of delivery, of which 5 ml was stored in a heparinized glass container for phthalates measurements as we have previously described [3], and the other 5 ml was used for assessing hormone and inflammatory mediators in serum. A section of placental tissue from each subject (at least 10 cm^3, sampled from the central area near the cord after serosa and amnion had been removed) was collected to measure PPARG protein expression.

2.2. Phthalates measurements

Phthalates in cord blood were extracted and measured as we have previously described [3]. Briefly, the samples were thawed and diluted with ultrapure water (1:1). The sample was extracted twice with 5 ml of hexane:MTBE (1:1) for 30 min and once with 3 ml hexane for 15 min. The extract was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS; Agilent 7890A\5975C; Agilent Technologies Inc., Santa Clara, CA, USA). Phthalates were identified and quantitated by their characteristic retention time, quantification ion and confirmation ion. At least two blanks (ultrapure water stored in a heparinized glass container at −80 °C) and two positive controls (ultrapure water containing the calibration standard stored in a heparinized glass container at −80 °C) were analyzed along with each batch of samples to monitor background concentrations of phthalates. A certified standard mixture of 15 phthalates (M-8061-R1; Accustandard Inc., New Haven, CT, USA) including Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP) and bis (2-ethyl hexyl) phthalate (DEHP) was used as a calibration standard.

2.3. Assessment of hormone and inflammatory mediators

Estriol (E3), estradiol (E2) and progesterone (P) in serum were assessed by radioimmunoassay with an automatic radioimmunoassay instrument (Anhui Ustc Zonkia scientific instruments co., LTD, Anhui, China) according to the manufacturer's instructions. Briefly, 100 μl of thawed serum samples and 1 ml of ^{125}I labeled substance was aliquoted into the coat-a-count tubes and incubated for 3 h at room temperature. The mixture was decanted and the tubes were counted for 1 min on a gamma counter. Samples and standards were analyzed in duplicate.

The inflammatory mediators matrix metalloproteinase (MMPs) was assessed in serum by ELISA method with commercially available kits (Beijing Cheng Lin biological technology co., LTD, Beijing, China) according to the manufacturer's instructions. Briefly, 100 μl of standards and thawed serum samples were incubated for 2 h at 37 °C in triplicate wells of an ELISA plate. After plate washing, biotinylated detection antibody was added to each well and the plate was incubated for 1 h at room temperature with gentle shaking. After a second wash, HRP-Streptavidin solution was added to each well and the plate was incubated for 45 min at room temperature with gentle shaking. After a third wash, the substrate reagent was added to each well and incubated for 30 min at room temperature in the dark with gentle shaking. Finally, the stop solution was added to each well and the plate was read at 450 nm immediately.

2.4. Protein assessment

Protein was extracted from placenta and the expression of PPARG was assessed by Western Blot as described previously [22].

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