Reproductive Toxicology 63 (2016) 142–150

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Effect of PFOS on glucocorticoid-induced changes in human decidual stromal cells in the first trimester of pregnancy

Qianlan Yang[a,b], Wangsheng Wang[a,c], Chao Liu[a,c], Yu Wang[b,*], Kang Sun[a,c,*]

[a] Center for Reproductive Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, PR China
[b] Department of Obstetrics and Gynecology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200135, PR China
[c] Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai, PR China

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 24 May 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Decidualization
PFOS
11β-HSD1
Cortisol
Proinflammatory cytokines

ABSTRACT

Perfluorooctane sulfonate (PFOS) is a surfactant and used in treating products for waterproofing and non-stick applications. PFOS has potential influence on reproductive function but the effect of PFOS exposure on the decidual functions remains unknown. By using primary human decidual stromal cells of early pregnancy we demonstrated that PFOS inhibited decidualization of the stromal cells as well as decidualization-induced upregulation of 11β-HSD1, an enzyme that regenerates biologically active cortisol from its inactive counterpart cortisone. Moreover, PFOS attenuated cortisol-induced decidualization and upregulation of 11β-HSD1 in the stromal cells. Furthermore, PFOS inhibited the reduction of IL-6 and IL-1β, the key proinflammatory cytokines in maternal-fetal immune intolerance, by cortisone in the decidual stromal cells indicating attenuated conversion of cortisone to cortisol. In conclusion, exposure to PFOS may disrupt the regeneration of cortisol in the decidual tissue thereby impairing the decidualization and immune-tolerance environment of early pregnancy.

1. Introduction

Because perfluorinated compounds are oil and water-resistant [1–4], they are widely used in treating products for waterproofing and non-stick applications including textiles, paper, leather, polishes, paints, cleaning products, carpets as well as metal surfaces and aqueous film forming foam for firefighting [5]. Perfluorooctane sulfonate (PFOS) is one of the most common perfluorinated compounds found in the environment [6]. PFOS accumulates in the human body with an average half-life of about 5.4 years [7]. In animal studies, PFOS can cause cancer, stunted growth, endocrine disruption and neonatal mortality [8]. In humans, high PFOS blood levels are associated with increased risks of chronic kidney and thyroid diseases, attention deficit hyperactivity [9–11]. Because of its potential harmfulness to human health, PFOS was classified as one of the persistent and ubiquitous organic pollutants at the Stockholm Convention in 2009 [12]. However the use of PFOS has not been completely phased out and it is still being produced in several countries [13]. With the growing global economy, consumer products containing PFOS may still impose a potential world-wide threat to human health.

Accumulating evidence has shown that PFOS exposure may have adverse effects on human gestation. Cohort analysis revealed that decreased fertility, increased risk of pre-eclampsia and prolonged gestation were associated with the plasma concentration of PFOS [14]. Furthermore, PFOS concentration in the cord serum correlated positively with the concentration in the maternal serum [15,16]. In utero exposure to PFOS was found to be negatively correlated with birth weight [17,18]. Recently we demonstrated that exposure to PFOS impaired human placental endocrine function by reducing the production of hormones crucial for the maintenance of pregnancy, such as human chorionic gonadotropin (hCG), progesterone and estradiol [19]. These findings indicate that PFOS exposure may jeopardize the establishment and maintenance of gestation. However, it remains unknown whether PFOS exposure has deleterious effects on decidual function thereby disrupting the establishment of pregnancy.

The decidual tissue of the human first trimester supplies a fertile and immune tolerance environment in the uterus for the early embryo, which is an important constituent of maternal and fetal interfaces in the establishment of pregnancy. The decidua

Abbreviations: PFOS, perfluorooctane sulfonate; 11β-HSD1, 11β - hydroxysteroid dehydrogenase 1; PFCs, perfluorinated compounds; cAMP, 8-bromo-cAMP; MPA, medroxyprogesterone acetate; PRL, prolactin; IGFBP, insulin growth factor binding protein 1; qRT-PCR, quantitative real time PCR; IL-1β, interleukin-1β; IL-6, interleukin-6.

* Corresponding authors at: Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200433, P.R.China.
E-mail addresses: renjiwangyu@gmail.com (Y. Wang), sungangrenji@hotmail.com (K. Sun).

http://dx.doi.org/10.1016/j.reprotox.2016.06.003
0890-6238/

expresses abundant amounts of glucocorticoid receptor [20,21] and 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) which regenerates biologically active cortisol from its inactive counterpart cortisone [22]. These findings highlight the importance of decidua as the glucocorticoid-target tissue in early gestation. Glucocorticoids have been demonstrated to be implicated in the induction of decidualization of the endometrial stromal cells [21] and the creation of intrauterine immune tolerance environment for the embryo by reducing the amount of uterine natural killer cells and the production of proinflammatory cytokines [23–25]. Therefore, appropriate amounts of glucocorticoids in the decidual tissue may underpin successful embryo implantation, placentation and early maintenance of gestation [26]. These beneficial actions of glucocorticoids advocate their use for the prevention of early pregnancy loss in clinical practice. Previous study has reported that 11β-HSD1 is highly up-regulated during decidualization [20], which indicates the important role of high levels of biologically active glucocorticoids in the decidua in early pregnancy. The aim of this study was to examine whether exposure to PFOS can have detrimental effects on decidualization and creation of the immune-competent environment via modulation of the expression of 11β-HSD1 and the actions of glucocorticoids in the decidua of early pregnancy.

2. Materials and methods

2.1. Tissue collection

Human first trimester (6–8 weeks) decidual tissue of normal pregnancy was obtained from women between 20 and 35 years old undergoing induced-abortion with written-informed consents in accordance with a protocol approved by the committee of ethical review of research involving human subjects, Renji Hospital, School of Medicine, Shanghai Jiao Tong University. Women with the history of spontaneous abortion and use of acyeterion (contraceptive containing estrogen or progesterone) were excluded from the study. Decidual tissue was processed for stromal cell isolation as described below.

2.2. Decidual stromal cell isolation and culture

Decidual stromal cells were isolated from the decidual tissue according to a previously described protocol [27] with slight modifications. Briefly, fresh decidual tissue was minced and subjected to 0.1% collagenase (type IV) (Sigma-Aldrich, St Lois, MO, USA) digestion in a shaking water bath at 37 °C for 60 min for two times. The tissue digest was then passed through a nylon sieve (38 μm) and the elute containing the stromal cells was centrifuged at 2500 rpm for 5 min at room temperature. After resuspension of the pellet, the cells were layered on a discontinuous (55%:50%:40%) Percoll (GE Healthcare Bio-Sciences, Uppsala, Sweden) gradients and centrifuged at 2500 rpm for 20 min at 4 °C. The top cell layer was collected and washed with serum-free DMEM F-12 (Gibco, Grand Island, NY) and centrifuged at 2000 rpm for 5 min at 4 °C. The cell pellet was resuspended and counted with a hemocytometer. Trypan blue exclusion test indicated that more than 95% of the isolated cells were viable. The stromal cells were plated at 1×10^6 cells per well in a 12-well plate or 2×10^6 cells per well in a 6-well plate for culture in DMEM F-12 (Gibco) containing 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotics (Gibco) at 37° in 5% CO_2/95% air.

2.3. Immunofluorescence staining of 11β-HSD1 in the decidual cells

To study the expression pattern of 11β-HSD1 during in vitro decidualization of the stromal cells prepared from the first trimester decidual tissue, immunocytofluorescence staining was carried out on the cells before and after induction with 0.5 mM 8-bromo-cAMP (cAMP) (Sigma) and 1 μM medroxyprogesterone acetate (MPA) (Sigma) in phenol red-free DMEM/F-12 (Gibco) containing charcoal-stripped FBS (Gibco) for 24 and 48 h. After fixing with 4% paraformaldehyde, the cells were permeablized with 0.4% Triton X-100 and then were incubated with antibodies against 11β-HSD1 (1:200) (Abcam, Cambridge, UK) overnight at 4 °C. Following washing with PBS, the cells were incubated with Alexa Fluor 488-labeled secondary antibody (green color) (1:100) (Proteintech, Chicago, USA) in darkness at room temperature for 2 h. The staining was examined under a fluorescence microscope (Zeiss, Germany). To identify the uterine epithelia and stromal cells, the cells were stained with antibodies against cytokeratin (1:100) (Santa Cruz) or vimentin (1:100) (Santa Cruz), markers for epithelial and stromal cells respectively, which showed that ≥99% of the cells were vimentin-positive (data not shown). Nuclei was counterstained with DAPI (blue color) (1 μg/ml). To test the specificity of immunohistochemical staining, cells were exposed to preimmune serum instead of the primary antibody and no staining was observed.

2.4. Treatment of the decidual stromal cells

To observe whether stromal cells from the first trimester can undergo further decidualization, 24 h after plating, the cells were treated with or without cAMP/MPA in phenol red-free DMEM/F-12 (Gibco) containing 2% charcoal-stripped FBS (Gibco) for 24 and 48 h. To examine the effect of PFOS on decidualization of the stromal cells, the cells were treated with PFOS (Sigma) in the presence and absence of cAMP/MPA induction two days after plating. Total RNA and protein were then extracted from the cells. The mRNA levels of prolactin (PRL) and insulin growth factor binding protein 1 (IGFBP1), markers of decidualization, were analyzed with quantitative real time PCR (qRT-PCR). To understand whether decidualization affects the expression of 11β-HSD1, 11β-HSD1 mRNA and protein levels were examined in these samples with qRT-PCR and Western blotting respectively. The effect of PFOS on the reductase activity of 11β-HSD1 was also examined as described below.

The influence of PFOS on cortisol-induced effects in the decidual stromal cells of the first trimester was also examined. Two days after plating, the cells were incubated with cortisol (Sigma) in the presence and absence PFOS for 24 h in phenol red- and serum-free medium. The cells were then collected to analyze PRL, IGFBP1, interleukin-1β (IL-1β), interleukin-6 (IL-6), 11β-HSD1 mRNA or protein abundance with qRT-PCR or Western blotting. Culture medium was collected to measure IL-1β and IL-6 with ELISA (R&D Systems, Minneapolis, MN) following the protocols provided by the manufacturer.

Because cortisone relies on 11β-HSD1 to be biologically active, we further tested whether exposure to PFOS diminished the effect of cortisone on IL-6 and IL-1β abundance; such an effect may indicate diminished conversion of cortisone to cortisol. The stromal cells were pretreated with PFOS for 24 h and then co-treated with PFOS and cortisone (Sigma) for 30 h. The cells and culture medium were collected to measure IL-6 and IL-1β abundance with qRT-PCR and ELISA.

2.5. Extraction of RNA and analysis with quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the cells after treatments using a total RNA kit (OMEGA Bio-Tek, Norcross, GA). RNA concentration and quality were determined by measuring OD260 and the ratio of OD260/OD280 with NanoDrop®ND-2000. Messenger RNA from the total RNA was reverse-transcribed to cDNA using PrimeScript® RT Master Mix Perfect Real Time kit (TaKaRa, Dalian, China). The