Toxicology Letters 286 (2018) 1–9

Contents lists available at ScienceDirect

Toxicology Letters

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

Course-, dose-, and stage-dependent toxic effects of prenatal dexamethasone exposure on fetal articular cartilage development

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ARTICLE INFO

Keywords:
Prenatal exposure
Dexamethasone
Developmental toxicity
Articular cartilage development
Transforming growth factor beta

ABSTRACT

Dexamethasone, a synthetic long-acting glucocorticoid, is routinely used for treating mothers at risk for preterm delivery. However, intrauterine overexposure to glucocorticoids induces low birth weight and cartilage dysplasia in offspring. Also, the "critical window" and safe dose of this treatment are largely unknown. This study investigated the course-, dose-, and stage-dependent toxic effects and the possible mechanisms of prenatal dexamethasone exposure (PDE) on fetal development and articular cartilage development. Pregnant mice (C57BL/6) received subcutaneous injection of dexamethasone (0.8 mg/kg d) once on gestational day (GD) 15 or once a day from GD 15 to 17, or received various doses of dexamethasone (0, 0.2, 0.8, and 1.2 mg/kg d) on GD 15–17, or received dexamethasone (0.8 mg/kg d) at early stage (GD 12–14) or late stage of pregnancy (GD 15–17). Offspring's knee joints were harvested at birth for morphological analyses and detection of gene expression. Repeated PDE significantly suppressed fetal and articular cartilage development, which were characterized by decreased body weight and body length, coarse articular cartilage surfaces, and reduced gene and protein expression of Col2a1 and aggrecan. For those newborns treated with repeated PDE at different doses, the toxic effects on fetal and articular cartilage development were observed at doses of 0.8 and 1.2 mg/kg d, whereas no obvious toxic effects were observed at the dose of 0.2 mg/kg d. Moreover, PDE at 0.8 mg/kg d during the early embryonic stage induced stronger toxic effects on fetal and articular cartilage development, compared with PDE during the late embryonic stage. Detection of gene expression showed that the TGFβ signaling pathway in the articular cartilage was down-regulated after PDE. Taken together, PDE induces fetal developmental toxicity and articular cartilage developmental toxicity in a course-, dose-, and stage-dependent manner.

1. Introduction

Dexamethasone, which is a synthetic long-acting glucocorticoid, has been widely used in preterm delivery to reduce the risk of neonatal respiratory distress syndrome (Crowther et al., 2015). However, with the widespread application of glucocorticoids in clinics, their toxic effect on embryonic development has been increasingly addressed. Clinical studies and animal experiments indicate that prenatal dexamethasone exposure (PDE) is associated with decreased birth weight and can induce intrauterine growth restriction (IUGR) (Murphy et al., 2008; Asztalos et al., 2014; Xu et al., 2011). IUGR refers to the growth restriction of embryos or fetuses that is caused by an adverse prenatal environment, and manifests as developmental disorders in multiple organs, growth retardation, and low birth weight (Fowden and Forhead, 2004). IUGR is diagnosed when the body weight of a fetus is below the 10th percentile for a given gestational age (Engelbregt et al., 2001). Increasing evidence suggests that fetuses with IUGR are susceptible to a variety of diseases in adulthood such as metabolic syndrome (Godfrey et al., 2011). Hence, fetal weight has become a "gold standard" for evaluating embryonic developmental toxicity. A comprehensive study of the influence of PDE on embryonic development can provide an experimental and theoretical basis for optimizing dexamethasone therapeutic regimens during pregnancy.

Increasing evidence suggests that articular cartilage develops

Abbreviations: PDE, prenatal dexamethasone exposure; GD, gestational day; TGFβ, transforming growth factor β; IUGR, intrauterine growth restriction; Col2a1, collagen type II alpha 1; RT-qPCR, real-time quantitative polymerase chain reaction; SE, single exposure; RE, repeated exposure; EE, exposure at early stage; EL, exposure at late stage; TGFβR1, transforming growth factor β receptor 1; Sox9, SRY-box 9; MOD, mean optical density; IOD, integrated optical density

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https://doi.org/10.1016/j.toxlet.2018.01.008
Received 23 September 2017; Received in revised form 17 December 2017; Accepted 9 January 2018
Available online 10 January 2018
0378-4274/

mainly during the embryonic period, and its development is closely associated with an embryo's overall development (Pitsillides and Beier, 2011). After articular cartilage matures, there is little growth and replication of chondrocytes; only in exceptional circumstances, such as trauma, osteoarthritis, and acromegaly, can reactive cell replication be observed, but the structure and function of these chondrocytes are quite abnormal (Dreier, 2010; Hayes et al., 2001). Therefore, the intrauterine developmental stage plays a decisive role in the structure and function of articular cartilage, as it directly and significantly impacts the quality of articular cartilage in adult offspring (Dreier, 2010). Epidemiological data have indicated that osteoarthritis is significantly associated with lower birth weights (Sayer et al., 2003; Jordan et al., 2005). Our laboratory conducted a series of animal experiments which demonstrated that fetal rats in which IUGR was induced by exposure to prenatal xenobiotics such as caffeine, nicotine, and alcohol showed increased susceptibility to osteoarthritis in adulthood (Ni et al., 2015; Luo et al., 2015; Tie et al., 2016). These results indicate that osteoarthritis may originate from the intrauterine developmental stage, and cartilage dysplasia during intrauterine development may predispose adult offspring to osteoarthritis. Up to now, how PDE will affect the intrauterine development of fetal articular cartilage is still unknown. Studying dexamethasone-induced fetal articular cartilage developmental toxicity, and investigating its mechanism, will be helpful for exploring new etiology and early prevention strategies for osteoarthritis.

According to the standard of practice, women at risk of preterm delivery at 24–34 weeks' gestation receive a single course of dexamethasone or betamethasone intramuscularly. It is recommended that women who are still pregnant 7–14 days later after the first course of prenatal corticosteroid therapy receive multiple courses of treatment to reduce the morbidity and mortality of preterm infants (Surbek et al., 2012); however, multiple courses of therapy are still controversial (Kemp et al., 2016). Clinic trials have demonstrated that repeated antenatal courses of dexamethasone significantly decrease fetal body weight, body length, and head circumference compared with the group that received a single course of dexamethasone (Murphy et al., 2012; Wapner et al., 2007). Therefore, the number of courses is a critical factor in determining the developmental toxicity of dexamethasone. Also, the dosage of dexamethasone treatment may be another important factor in mediating its developmental toxicity. There is growing evidence that current corticosteroid dosing regimens may result in adverse outcomes during pregnancy. However, even the most well-established antenatal steroid therapies lack the comprehensive pharmacokinetic and dose–response data necessary to optimize dosing regimens (Kemp et al., 2016). Moreover, the toxic effect of PDE is also associated with exposure during the embryonic stage. Animal experiments have demonstrated that there are "critical window" for the toxic effect of PDE. This time window appears to be organ-specific, and it might coincide with the specific period for embryonic organ development (LaBorde et al., 1992; Carlos et al., 1992; Miller and Witchel, 2013). Hence, PDE has course-, dose-, and gestational stage-dependent effects on fetal organ development. Despite all these, the course-, dose-, and stage-dependent effects of PDE on fetal development and articular cartilage development remain unclear.

In the present study, using the established PDE mouse model, we aimed to demonstrate the effects of PDE on fetal development and articular cartilage development and analyze its course-, dose-, and stage-dependent effects. We also sought to investigate the effect of PDE on the transforming growth factor β (TGFβ) signaling pathway, which is closely related to cartilage matrix synthesis.

2. Materials and methods

2.1. Drugs and reagents

Dexamethasone (CAS. NO. 50-02-2, 1 ml: 5 mg, AR) was obtained from Kingyork Group Co., Ltd. (Tianjin, China). Safranin-O (CAS. NO. 477-73-6) was obtained from Hengyuan Biotech Co., Ltd. (Shanghai, China). Monoclonal mouse collagen type II alpha 1 (Col2a1) antibody (sc-52658, 100 μg/ml) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Trizol reagent kits were obtained from Omega Bio-Tek (Doraville, USA). The SYBR Green dye was purchased from ABI (Foster City, CA, USA). Reverse transcription and real-time quantitative polymerase chain reaction (RT-qPCR) kits were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The oligonucleotide primers for mouse RT-qPCR genes (PAGE purification) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Other chemicals and agents were of analytical grade.

2.2. Animals and treatments

Specific pathogen-free C57BL/6 mice weighing 19–21 g (53–57 days old, female) and 22–24 g (45–63 days old, male) were obtained from the Experimental Center of the Hubei Medical Scientific Academy (No. 2015-0018, Wuhan, China). Animal experiments were performed in the Center for Animal Experiment of Wuhan University (Wuhan, China), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The Committee on the Ethics of Animal Experiments of the Wuhan University School of Medicine approved the protocol (permit number: 14016). All animal experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee. Animals were housed in metal cages bedded with corn cob in an air-conditioned room under standard conditions (room temperature: 18–22 °C; humidity: 40%–60%; light cycle: 12 h light-dark cycle; 10–15 air changes per hour) and allowed free access to mouse chow and tap water. All mice were acclimated one week before experimentation. For mating, two female mice were placed together with one male mouse overnight in a cage. The appearance of vaginal plug confirmed mating, and the day of mating was taken as gestational day (GD) 0. Pregnant mice were housed individually in cages bedded with corn cob. Food and water were freely available.

When pregnant mice were grouped into different treatment groups, the two female mice breed to the same male mouse were not allowed to be assigned to the same group. To evaluate the effects of different courses of PDE on fetal development and articular cartilage development, pregnant mice were received subcutaneous dexamethasone (0.8 mg/kg body weight) or vehicle (the same amount of normal saline) injection once every day (between 8 AM and 9 AM) on GD15 for single exposure (SE) group, or from GD 15 to 17 for repeated exposure (RE) group. To characterize the dose-dependent effect of dexamethasone, pregnant mice in the RE group were treated with various doses of dexamethasone (0, 0.2, 0.8, and 1.2 mg/kg d) once a day from GD 15 to 17. The vehicle group received daily subcutaneous injections of normal saline during the same period. In addition, to examine the stage-dependent effects of PDE on both fetal and articular cartilage development, pregnant mice in the exposure at early stage (EE) group were treated with dexamethasone (0.8 mg/kg d) once a day from GD 12 to 14, and pregnant mice in the exposure at late stage (EL) group were treated once a day from GD 15 to 17, respectively. Pregnant mice in each group were maintained until normal delivery (n = 6/group). Mice offspring in each group were euthanized on the birth day. The body weight and body length were then recorded. IUGR was diagnosed when the body weight of an animal was two standard deviations lower than the mean body weight of the control group (Engelbregt et al., 2001). Two newborn mice (one male and one female) were selected randomly from every litter. Newborns' right knee cartilage was separated under a dissection microscope and then collected. The collected samples were immediately frozen in liquid nitrogen, followed by storage at −80 °C for subsequent RT-qPCR analyses. Newborns' left knee joints were separated and fixed in 4% paraformaldehyde for 24 h before being embedded in paraffin for further analyses.