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Prenatal glucocorticoid contributed to rat lung dysplasia is related to asymmetric dimethylarginine/nitric oxide pathway

Yu-Chieh Chen · Li-Tung Huang · You-Lin Tain · Chih-Cheng Chen · Jiunn-Ming Sheen · Mao-Meng Tiao · Chih-Min Tsai · Ho-Chang Kuo · Chao-Cheng Huang · Kow-Aung Chang · Hong-Ren Yu

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Abstract Prenatal glucocorticoids (GCs) have been used to induce maturation of preterm fetal lungs and prevent the development of respiratory distress syndrome of the premature. Pulmonary surfactant induction has been regarded as the most important effect of prenatal GCs. However, report about the prolonged effects of prenatal GCs on the development of rat lung is of limited. In this study, we tried to investigate the acute and chronic modulation effects of prenatal dexamethasone (DEX) to asymmetric dimethylarginine (ADMA)/nitric oxide (NO) signal pathway of lung tissue. Pregnant Sprague Dawley rats at gestational day 14–20 were administered i.p. DEX (0.1 mg kg^{-1} d^{-1}). Acute programming effects of prenatal DEX were assessed at postnatal day 7, and long-term programming effects of offspring were assessed at day 120. We found that repetitive prenatal DEX exposure contributes to DNA oxidative damage and alveolar tissue dysplasia. Prenatal DEX treatment decreased ADMA and increased iNOS expression. Prenatal DEX treatment also increased TNF-α transcript expression and decreased HDAC2 protein expression at acute stage. In conclusion, repetitive prenatal DEX has prolonged stress damage effects on lung tissue.

Keywords Prenatal glucocorticoids · ADMA · DNA oxidative damage · TNF-α · HDAC2 · Lung dysplasia

1 Introduction

Lung immaturity contributes to the major cause of the mortality and morbidity of preterm baby. Prenatal glucocorticoids (GCs) have been used to induce maturation of preterm fetal lungs and prevent the development of respiratory distress syndrome (RDS) of the premature [1]. Surfactant induction [2], lung growth enhancement [3], pulmonary epithelial cells maturation and differentiation [4] are well-known effects of prenatal GCs. Inhibition of DNA synthesis, decrease in cell proliferation and thus enhancement of cell differentiation by prenatal GCs are thought to be the mechanism for fetal lung maturation [5].

Nitric oxide (NO) has multiple effects on lung growth [6]. NO involves in angiogenesis, airway smooth muscle proliferation and even branching morphogenesis of the lung [7]. Through NO synthase (NOS), NO is generated from L-arginine. Arginine analogs asymmetric dimethylarginine (ADMA) and NG-monomethyl L-arginine (L-NMMA) can competitively inhibit the NO synthesis process. Both ADMA and L-NMMA are products of proteolytic degradation of methylated proteins. Other GC effects on lung tissue maturation include induction of antioxidant enzymes and endothelial NOS (eNOS) [8].

Y.-C. Chen · L.-T. Huang · Y.-L. Tain · C.-C. Chen · J.-M. Sheen · M.-M. Tiao · C.-M. Tsai · H.-C. Kuo · H.-R. Yu (✉)
Department of Pediatrics, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Graduate Institute of Clinical Medical Science, Chang Gung University College of Medicine, Kaohsiung, Kaohsiung
e-mail: yuu2004taiwan@yahoo.com.tw

C.-C. Huang
Department of Pathology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Graduate Institute of Clinical Medical Science, Chang Gung University College of Medicine, Kaohsiung, Kaohsiung

K.-A. Chang
Department of Anesthesiology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Graduate Institute of Clinical Medical Science, Chang Gung University College of Medicine, Kaohsiung, Kaohsiung

However, GCs can also result in overproduction of reactive oxygen species and dysregulation of physiological processes in certain conditions [9]. With repeated use, prenatal GCs may cause significant growth restriction and neurodevelopmental handicaps [10]. These potential adverse effects have restricted the application of prenatal GCs for fetal lung maturation.

It had been reported that prenatal exposure to dexamethasone causes birth weight reduction in rats [11]. Liver, visceral adipose tissue, skeletal muscle, pancreas and brain are reported to be the major target organs of GC programming [12]. With the exposure of prenatal steroid, offspring even expresses hypertension, hyperglycemia, anxiety and increased hypothalamic–pituitary–adrenal axis activity at adult stage [11]. However, reports about the prolonged effects of prenatal GCs to the development of lung are very limited. In this study, we attempted to investigate the acute and chronic modulation effects of prenatal GCs on lung tissue, including the ADMA/NO signal pathway.

2 Materials and methods

2.1 Animals

Sprague Dawley (SD) rats were housed in the animal care facility in Chang Gung Memorial Hospital. All animal experiments were performed in accordance with legislation on the protection of animals and were approved (Permit number: 2012022304) by the animal care committee at Chang Gung Memorial Hospital. All procedures were performed under anesthesia, and all efforts were made to minimize suffering. SD female rats were allowed to mate with male rats for 24 h. One day later, female rats were separated from the male rats and housed individually in a standard plastic home cage. After confirmation of pregnancy on the 14th day after mating, pregnant females were randomly divided for the prenatal GC exposure paradigm, or left undisturbed until delivery. The pregnant rats were checked for litters daily. The day of birth was designated postnatal day 0 (D0). Rat pups were weaned at postnatal day 21 (D21) and had access to standard chow and water ad libitum. Only male rats were used in this study.

Pregnant SD rats at gestational day (GD) 14–20 were administered i.p. dexamethasone (0.1 mg kg^{-1} d^{-1}). Acute effects by GC were assessed at postnatal day 7 (D7) rat, and long-term programming effects were assessed at postnatal day 120 (D120). The sham group was pregnant SD rats at GD 14–20 administered i.p. normal saline [12].

2.2 Histologic examination

The rats were killed, and the lungs were harvested and stored in saline on ice and dissected from the surrounding tissues. Then, the lung tissues were fixed in 10 % formalin neutral buffer solution, pH 7.4 (Wako Junyaku, Osaka, Japan). The paraffin-embedded lung tissues were cut at 4 μm and stained by hematoxylin and eosin (H&E). The slides were evaluated by light microscope at a magnification of ×200 by a pathologist.

2.3 Western blotting

Each 50 mg lung tissue sample was homogenized with 500 μL PRO-PREP Protein Extraction Solution (#17081, iNtRon Biotechnology, Korea), and cells were lysed by incubation for 30 min on ice and then centrifuged at 14,000 ×*g* for 20 min at 4 °C. Protein concentrations were determined by using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Protein samples (50 μg) were boiled with gel-loading buffer for 5 min and then subjected to 10 % SDS-PAGE. After transfer to a polyvinylidene fluoride membrane (Roche Applied Sciences, Basel, Switzerland) and block with PBS–Tween containing 5 % dry milk, the membranes were then incubated for 2 h with the following anti-rat antibodies: inducible NOS (iNOS), eNOS (#610297, BD transduction Laboratories, San Diego, CA, USA), NADPH oxidase (Sigma, St. Louis, MO, USA) diluted 1:200 in TBS containing 1 % skim milk. After five washes with 0.1 % T-TBS, the membranes were incubated for 1 h with peroxidase-labeled secondary antibody diluted 1:1,000 in T-TBS. After rinsing with T-TBS, the membranes were developed using Western Lightning Plus-ECL (NEL105001EA, Perkin-Elmer, Waltham, USA) for Kodak X-ray film exposure.

2.4 Real-time quantitative RT-PCR

In brief, total RNA was extracted from the lung tissue with a Trizol reagent (#15596-018, Invitrogen, Carlsbad, CA, USA). A 5-μg sample of total RNA was reversed-transcribed with 200 U of M-MLV Reverse Transcriptase (#28025-021, Invitrogen) in 20 μL of total reaction volume containing reverse transcriptase buffer, random primer, dNTP and RNase inhibitor. PCR was performed in 20 μL of total reaction volume containing 2 μg of cDNA, primers specific for TNF-α, IL-8, GM-CSF and MMP-9, 2.5 mmol L^{-1} $MgCl_2$ and Maxima SYBR Green/Fluorescein qPCR Master Mix (2X) (#K0242, Thermo Scientific, California, USA). The cycling protocol consisted of one cycle of 10 min at 95 °C, followed by 45 cycles of denaturation for 10 s at 95 °C, annealing for 20 s at 55 °C and extension for