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Protective effect of electro-acupuncture at maternal different points on perinatal nicotine exposure-induced pulmonary dysplasia in offspring based on HPA axis and signal transduction pathway

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ABSTRACT

Perinatal nicotine exposure can not only lead to lung dysplasia in offspring, but also cause epigenetic changes and induce transgenerational asthma. Previous studies have shown that electro-acupuncture (EA) applied to "Zusanli" (ST 36) can improve the lung morphology and correct abnormal expression of lung development-related protein in perinatal nicotine exposure offspring. However, it is still unclear whether ST 36 has a specific therapeutic effect and how maternal acupuncture can protect the offspring from pulmonary dysplasia. In this study, we compared the different effect of ST 36 and "Fenglong" (ST 40), which belong to the same meridian, in terms of lung pulmonary function and morphology, PPARγ, β-catenin, GR levels in the lung tissues and CORT in the serum of perinatal nicotine exposure offspring, and explored the mechanism of acupuncture based on the maternal hypothalamus-pituitary-adrenal (HPA) axis. It is shown that EA applied to ST 36 could restore the normal function of maternal HPA axis and alleviate maternal glucocorticoid overexposure in offspring, thereby it can up-regulate the PTHrP/PPARγ and down-regulate the Wnt/β-catenin signaling pathways, and protects perinatal nicotine exposure-induced pulmonary dysplasia in offspring. Its effect is better than that of ST 40. These results are of great significance in preventing perinatal nicotine exposure-induced pulmonary dysplasia in offspring.

1. Introduction

Although there are evidences that maternal smoking is associated with adverse outcomes such as premature birth, low birth weight, stillbirth and fetal mortality et al. [1,2], many women still smoke actively or passively during the perinatal period [3,4]. Nicotine can be absorbed quickly and accumulated in the fetus through the placenta or breast milk and it is considered to be the main toxic component of tobacco harmful to fetal health [5,6]. Therefore, smoking during perinatal period can cause nicotine accumulation in the fetus or neonate, which impacts the development of multiple systems [7–17], especially the respiratory system. Previous studies have revealed that perinatal nicotine exposure has adverse effect on the lung function and structure of the offspring [9–11], making the offspring susceptible to asthma, emphysema and other respiratory diseases [12–14], and further increases the risk of asthma in the third generation [15,16].

The PTHrP/PPARγ signaling pathway is involved in perinatal nicotine exposure-induced pulmonary dysplasia in offspring. In physiological state, the moderate tension of alveolar type II cells can promote the secretion of parathyroid-hormone-related protein (PTHrP) and Prostaglandin E2 (PGE2). PTHrP stimulates uptake of neutral lipid by lung fibroblasts, which turn to lipofibroblasts. After PTHrP binds to lipofibroblast receptors and the process of upregulation of PPARγ via Protein Kinase A activation (PKA), PPARγ promotes the expression of downstream Adipocyte-Differentiation-Related Protein (ADRP), stimulates the uptake of triglyceride (TG) by the lipofibroblasts and ATII cells and induces lipofibroblasts to produce leptin. When leptin binds to alveolar type II cells, surfactant is produced to maintain normal lung structure and function [17]. Down-regulation of the expression of PPARγ will cause the transdifferentiation of lipofibroblasts to myofibroblasts,

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which cannot support the growth and differentiation of type II cells and therefore cannot produce surfactants, thus inhibiting normal lung development [17]. Previous studies have shown that perinatal nicotine exposure reduced the expression of PPARγ and ADRP in the second generation [18]. However, PPARγ agonists could completely block the change of the lung function and airway contraction markers induced by nicotine [19], which indicates that down-regulation of PTHrP/PPARγ is one of the pathways inducing pulmonary dysplasia in perinatal nicotine exposure.

The Wnt/β-catenin signaling pathway also plays an important role in perinatal nicotine exposure induced-pulmonary dysplasia. It is the key determinant of myofibroblast differentiation [20] and has a great impact on distal lung development, formation of tracheal branches and proliferation and differentiation of bronchial epithelial cells. It has been found that pretreatment of fibroblasts with nicotine could up-regulate the expression of the nicotinic acetylcholine receptors (nAChR), activate PKC and Wnt signals and then cause the transdifferentiation of lipofibroblasts to myofibroblasts. But PPARγ inhibitors could block nicotine-mediated Wnt activation [21]. Other experiments showed that the levels of β-catenin and LEF-1 in the lung of perinatal nicotine exposure rats were higher than those in the normal group, but this change could be reversed by the PPAR- γ agonist [22], indicating that the effects of perinatal nicotine exposure on the second generation were related to the Wnt signaling pathway, and the down-regulation of PPARγ affected this pathway.

In addition, neuroendocrine factors might also be involved in perinatal nicotine exposure-induced pulmonary dysplasia in offspring. Glucocorticoid, as the main end product of (HPA) axis, plays an important role in the development of fetal lung, which can participate in lung morphogenesis, promote lung maturation, and induce the synthesis and secretion of surfactant in alveolar type II cells [23]. However, excessive glucocorticoids can cause adverse effects on the lung development. Researches show that excessive glucocorticoids can lead to dysplasia of the alveolar structure and increase incidence of emphysema [24]. Studies also show that prenatal nicotine exposure can increase the expression of StAR and P450scc in maternal adrenals, decrease the expression of StAR and P450scc in fetal adrenals and inhibit the inactivation of maternal glucocorticoid in placenta. This can lead to maternal glucocorticoid overexposure, characterized by increased corticosterone in maternal and fetal serum and abnormal neuroendocrine metabolism associated with HPA axis [25,26]. Previous studies have shown that perinatal nicotine exposure can lead to increased glucocorticoid receptors (GR) in lung tissues of neonatal rats, and maternal EA applied to ST 36 can restore it to normal [18]. Therefore, perinatal nicotine exposure-induced pulmonary dysplasia in offspring may be related to maternal glucocorticoid overexposure.

Acupuncture can enhance body's self-regulation function, which can be used to treat respiratory diseases [27,28] and regulate the HPA axis [28,29]. In ancient records and modern researches, it has been found that ST 36 is an important acupoint in prevention and treatment of diseases and improvement of health by regulating the body's own function. It is also used in the treatment of asthma, chronic obstructive pulmonary disease, pulmonary fibrosis and so on [30–32]. Previous studies have shown that EA applied to maternal ST 36 can improve the lung morphology and correct abnormal expression of lung development-related protein in perinatal nicotine exposure offspring [18]. However, it has not been reported whether ST 36 has a specific therapeutic effect and how maternal acupuncture can protect the offspring from pulmonary dysplasia, which is worthy of further study.

In this study, we would compare the different effect of ST 36 and ST 40, which belong to the same meridian, in terms of lung development in perinatal nicotine exposure offspring based on PTHrP/PPARγ and Wnt/β-catenin signaling pathways. We also explored whether EA could protect the offspring from perinatal nicotine exposure induced pulmonary dysplasia by regulating the maternal HPA axis and reducing maternal glucocorticoid overexposure.

2. Materials and methods

2.1. Animals

Twenty-four female and eight male SPF grade adult (11-week old) Sprague-Dawley rats without mating history were acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Production license number: SCXK (Beijing) 2006–009). The rearing conditions were room temperature (23 ± 1) °C, relative humidity (45 ± 5) %, and they were maintained on a 12-h light: 12-h dark cycle. All animal procedures were in compliance with the Guide for Care and Use of Laboratory Animals advocated by the NIH and were approved by the Ethics Committee of Beijing University of Chinese Medicine (approval number: 3-2017020921-1021).

2.2. Experimental protocol

After the acclimatization period of one week, the female rats were randomly divided into 4 groups: saline group (S Group), nicotine group (N Group), nicotine plus EA ST 36 group (N + ST 36 Group), nicotine plus EA ST 40 group (N + ST 40 Group), 6 rats in each group. Three female rats randomly cohabitated with one male rat in the evening, and the vaginal smear was observed under a microscope on the second morning. The day when sperm was discovered was considered as the embryonic day (ED) 0. Pregnant rats were administered saline injection (S group), nicotine injection (N group), nicotine injection plus EA ST 36 treatment (N + ST 36 group), or nicotine injection plus EA ST 40 treatment (N + ST 40 group) from ED 6 to postnatal day 21 (PND 21) (the delivery day was skipped). Nicotine (Sigma) was injected subcutaneously, 1 mg/kg/day in 100 μl volume and saline was injected subcutaneously in 100 μl volume once a day. On PND 21, the lung function test was first performed and then the lung tissues and serums of the offspring and the maternal pituitaries, adrenal glands and serum were collected for ELISA or Real-time PCR testing.

2.3. Electro-acupuncture treatment

The selection of ST 36 and ST 40 is referred to *Experimental Acupuncture Science*. ST 36 is located at the posterolateral part of the knee joint, about 5 mm below the fibulae capitulum, while ST 40 is located at the midpoint of the connecting line between the popliteal stripe and the lateral malleolus tip, 2 mm from the lateral tibia. Acupuncture needles, 0.20 mm × 13 mm in size, were perpendicularly inserted into bilateral ST 36 or ST 40 points (connecting the negative pole) at a depth of 7 mm and horizontally inserted into the skin 2 mm below the acupoint (connecting the positive pole). 1 mA electric current with 2/15 Hz frequency was applied for 20 min. As stated above, EA was applied once every day from ED6 to PND 21.

2.4. Pulmonary function testing

The neonatal rats were anesthetized with 2% pentobarbital sodium (55 mg/kg body weight), tracheostomized, and a cannula was placed and fixed. Then the rats were placed in plethysmograph and connected to a ventilator. Forced vital capacity (FVC) was measured after recording a period of calm breath. The peak expiratory flow (PEF), lung resistance (RL), dynamic compliance (Cdyn) and FVC

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