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Low-expressional IGF1 mediated methimazole-induced liver developmental toxicity in fetal mice

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ABSTRACT

Anti-thyroid drugs (ATDs) therapy is necessary for pregnant women with hyperthyroidism. However, there is a lack of studies on developmental toxicity of ATDs. In this study, we observed the developmental toxicity of fetal liver induced by prenatal methimazole exposure (PME) in mice, and explored the potential mechanism. Pregnant Kunming mice were administered intragastrically with 4.5 or 18 mg/kg·d methimazole from gestational day (GD) 9～18. After PME, the birth weights of the offspring mice were decreased, and the liver morphology, development indexes and metabolic function were all altered in different degree in the PME fetuses. Meanwhile, PME decreased the levels of serum and hepatic insulin-like growth factor 1 (IGF1), and reduced the gene expression of IGF1 downstream signaling pathway. Furthermore, the protein levels of phosphorylated-extracellular regulated protein kinases (p-ERK) and serine-threonine protein kinase (p-Akt) were also reduced. Furthermore, methimazole disturb hepatocyte differentiation, maturation and metabolic function through suppressing IGF1 signaling pathway in HepG2 cells. These results demonstrated that PME could induce fetal liver developmental toxicity, and the underlying mechanism was related to low-expression of hepatic IGF1 caused by methimazole, which mediated abnormal liver morphology and metabolic function.

1. Introduction

Epidemiological investigation indicates that morbidity of hyperthyroidism in pregnant women is approximately 1‰–2‰ (Cooper and Laurberg, 2013). Hyperthyroidism during pregnancy is associated with the increased risk of abortion, premature birth, intrauterine growth retardation (IUGR), stillbirth, maternal hypertension, and thyroid storm (Krassas et al., 2010). Although anti-thyroid drugs (ATDs), radioiodine (I^{131}) and surgery are all medical therapies used to treat hyperthyroidism, radioiodine therapy is contraindicated during pregnancy, and surgery is often associated with risk of abortion. Therefore, ATDs are the preferred treatment for hyperthyroidism during pregnancy (Chan and Mandel, 2007). Methimazole and propylthiouracil are commonly used ATDs in hyperthyroidism treatment during pregnancy, and methimazole is mainly used during the second and third trimester (Hackmon et al., 2012). Therefore, it is necessary to study the developmental toxicity of ATDs and provide guidance for the safety of ATDs medication during pregnancy.

Methimazole can pass through placenta and its concentrations in fetal circulation are 30%–80% of maternal levels (Clark et al., 2006). Clinical research reported the birth defects in children born from mothers who took methimazole during pregnancy, and indicated a teratogenic potential of methimazole (Bahn et al., 2009; Endocrine et al., 2007). Other studies suggested that prenatal exposure to methimazole is associated with many congenital anomalies, for instance, choanal atresia, aplasia cutis, esophageal atresia, facial abnormalities, hematuria, and cardiovascular defects (Aramaki et al., 2005; Barbero et al., 2004, 2008; Clementi et al., 2010). But some large cohort studies indicated that birth defects, which associated with maternal hyperthyroidism, were attributed to thyroid dysfunction rather than ATDs (Di

Abbreviations: IGF-1R, insulin-like growth factor-1 receptor; Akt2, serine-threonine protein kinase 2; HMGCR, HMG CoA reductase; FASN, fatty acid synthase; PEPCK, phosphoenolpyruvate carboxykinase; PCNA, proliferating cell Nuclear antigen; HNF4α, hepatocyte nuclear factor 4α; ALB, albumin; AFP, alpha fetoprotein; GAPDH, glyceraldehyde phosphate dehydrogenase; Akt, serine-threonine protein kinase; p-Akt, phosphorylated Akt; ERK, extracellular regulated protein kinase; p-ERK, phosphorylated ERK; GD, gestational day; IGF1, insulin-like growth factor 1; IUGR, intrauterine growth retardation; PME, prenatal methimazole exposure; ATDs, anti-thyroid drugs

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Gianantonio et al., 2001; Momotani et al., 1984; Vandijke et al., 1987). Due to the potential teratogenicity of methimazole, it is recommended to use in the second and third trimester (De Groot et al., 2012). However, there have been few studies on the potential hepatotoxicity of methimazole in developing embryo during the second and third trimester.

Drug developmental toxicity refers to any structural or functional alteration caused by drugs, including structural malformation, dysfunction, growth retardation and even death. As the most common outcome of developmental toxicity, IUGR refers to the poor growth of a baby in the womb during pregnancy, and may lead to multiple organ dysplasia, growth retardation and low birth weight (Imdad et al., 2011). Insulin like growth factor 1 (IGF1) signaling pathway, which is the core of endocrine system, is involved in proliferation, differentiation, metabolism and matrix synthesis (Randhawa and Cohen, 2005). In regulation of metabolism, IGF1 has insulin-like effects and mediates the glucose and lipid metabolism (Sutter et al., 2007). In the early stage of development, liver is the primary source of IGF1 in circulation, and IGF1 autocrine was gradually established in extra-hepatic organs in the third trimester (Dercole et al., 1980). It was confirmed that the abnormal expression of IGF1 gene played a vital role in IUGR induced by prenatal adverse environment (Fall et al., 1995; Kamei et al., 2011). All above findings suggested that as a critical factor mediates the occurrence of IUGR, abnormal expression of IGF1 might mediate many metabolic diseases in offspring with IUGR.

To explore the developmental toxicity of fetal liver induced by prenatal methimazole exposure (PME), we treated the pregnant mice with methimazole during middle and late gestation, observed the birthweight, fetal liver development, and glucose and lipid metabolic function. Moreover, we verified the role of IGF1 signaling pathway in PME-induced liver developmental toxicity in HepG2 cells. This study may help us understand developmental toxicity of methimazole, and provide reference for guiding the safety use of methimazole during pregnancy.

2. Materials and methods

2.1. Materials

Methimazole (CAS no. 60-56-0) was obtained from Sigma-Aldrich Co., Ltd (St Louis, MO, USA). Isoflurane was obtained from Baxter Healthcare Co. (Deerfield, IL, USA). IGF1 ELISA kit was obtained from Assaypro LLC. (Saint Charles, Missouri, USA). Glucose oxidase assay kit was provided by Shanghai Mind Bioengineering Co., Ltd. (Shanghai, China). Triglyceride (TG) and total cholesterol (TCH) assay kits were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Primary antibodies such as rabbit anti-IGF1, rabbit anti-serine-threonine protein kinase (Akt), rabbit anti-phosphorylated Akt (p-Akt), rabbit anti-extracellular regulated protein kinase (ERK), rabbit anti-p-extracellular regulated protein kinase (p-ERK) and rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The enhanced chemiluminescence kit (ECL) was obtained from Pierce Biotechnology Inc. (Rockford, IL, USA). Reverse transcription and real-time quantitative PCR (RT-qPCR) kits were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). All oligonucleotide primers of RT-qPCR genes (PAGE purification) were custom synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Trizol reagent, Phosphate buffer saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, streptomycin, penicillin and trypsin were purchased from Thermo Fisher Scientific (Waltham, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay kit was purchased from Promega Biotech Co. (Madison, WI, USA). RIPA lysis buffer and BCA protein assay kit were obtained from Beyotime (Shanghai, China). All other chemicals and reagents were of analytical grade.

2.2. Animals and treatments

Specific pathogen free (SPF) Kunming mice (female body weight: 20 ~ 30 g; male body weight: 25 ~ 35 g) were purchased from the Experimental Center of Hubei Medical Scientific Academy (No. 2017-0018, certification number: 42000600014526, license number: SCXK (Hubei). Kunming mice, which is derived from Swiss mouse, are the most widely studied strain in China (Zhang et al., 2007). Animal experiments were performed in the Center for Animal Experiments of Wuhan University (Wuhan, China), which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). All animal experimental procedures were approved by and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee. All animal experimental procedures were approved by the Ethics of Animal Experiments Committee of Wuhan University, School of Medicine (Permit Number: 14,016). All mice were acclimated one week before experimentation, and one female mouse were housed with one male mouse overnight for mating. The appearance of sperm in vaginal smears was identified as mated, and the day of mating was taken as gestational day (GD) 0.

The mice treated with methimazole were divided into low-dose (ML) and high-dose group (MH), and intragastrically administered 4.5 or 18 mg/kg methimazole from GD9-18 once per day, respectively (the dosage setting was descripted in the Discussion section). The animals in control group were given the same volume of distilled water. On GD18, about 2 h after drug administration, the pregnant mice were anesthetized by isoflurane and then sacrificed. Since litter size is mostly distributed in 10 to 16, we kept fetuses from litter size from 10 to 16 for further study, and excluded the litter size fewer than 10 or more than 16. The sample size n = 10, represents numbers of mother. Each fetus was weighed after being dried on filter papers. IUGR pup is defined as a fetus weighing two standard deviations less than the mean body weight of control fetuses (Engelbregt et al., 2001). Fetal blood samples were collected and the serum was isolated. Fetal livers from each littermate were immediately separated and frozen in liquid nitrogen, followed by storage at −80 °C for subsequent analysis.

2.3. Cell culture and treatment

HepG2 cells were cultured in DMEM medium (pH 7.2–7.4) containing 10% fetal bovine serum, 100 IU/mL penicillin and 100 μg/ml streptomycin under 37 °C homothermal condition with 5% CO_2. The cells were cultured with non-serum medium overnight before experiments. After treatment with different concentrations of methimazole (0.8, 4, 20 μM) and/or IGF1 for 24 h, the cells were harvested for further analysis.

2.4. Cell supernatant IGF1 and serum-related biochemical detection

The concentrations of cell supernatant IGF1, serum IGF1, glucose, TG and TCH were measured by biochemical assay kits following the manufacturer's protocols (Luo et al., 2014).

2.5. Histological examination

The livers were fixed in a 4% paraformaldehyde solution and processed using the paraffin section technique. Sections were approximately 5-μm thick, stained with hematoxylin and eosin (HE), and observed under a light microscope.

2.6. Total RNA extract and RT-qPCR

Hepatic tissue or HepG2 cells were homogenized in Trizol reagent. The total RNA was extracted according to the manufacturer's protocol. The concentration and purity of the isolated total RNA were determined