Molecular and Cellular Endocrinology 348 (2012) 120–127

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Treatment with a constitutive androstane receptor ligand ameliorates the signs of preeclampsia in high-fat diet-induced obese pregnant mice

H. Masuyama *, Y. Hiramatsu

Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Department of Obstetrics and Gynecology, Okayama, Japan

ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 24 July 2011
Accepted 26 July 2011
Available online 4 August 2011

Keywords:
Adipocytokine
Constitutive androstane receptor
Insulin resistance
Preeclampsia

ABSTRACT

Constitutive androstane receptor (CAR) has been reported to decrease insulin resistance, while obesity and insulin resistance may also be involved in the pathogenesis of preeclampsia. We examined whether a CAR ligand, 1,4-bis(2-(3,5-dichloropyridyloxy)) benzene (TCPOBOP), can ameliorate the signs of preeclampsia in high-fat diet (HFD)-induced obese pregnant mice to examine a possibility of CAR as a therapeutic target. We employed six groups including non-pregnant, HFD-fed or control diet-fed pregnant mice with or without TCPOBOP treatment ($n = 6$). In HFD pregnant mice, insulin resistance increased with increasing expression of gluconeogenic and lipogenic genes and abnormal adipocytokine levels. TCPOBOP treatment, which was once-weekly intraperitoneal injections (0.5 mg/kg) and started at day 0.5 of pregnancy, improved glucose tolerance with significant changes of gluconeogenic, lipogenic and adipocytokine genes. HFD pregnant mice had hypertension and proteinuria, while TCPOBOP treatment ameliorated these signs. Our data suggested CAR might be a potential therapeutic target for obese preeclampsia patients with insulin resistance.

1. Introduction

Preeclampsia is characterized by the onset of high blood pressure and proteinuria. It occurs in about 3–5% of all pregnancies and results in substantial maternal and neonatal morbidity and mortality (Cunningham et al., 2001; Sibai et al., 2005). Type 1 and type 2 diabetes, gestational diabetes and polycystic ovarian syndrome are also well known risk factors for preeclampsia (Seely and Solomon, 2003). Furthermore, insulin resistance is implicated in the pathophysiology of preeclampsia and has been observed in some patients before the onset of preeclampsia (Innes et al., 2001; Wolf et al., 2002). Several reports have demonstrated that an increased body mass index (BMI) increases the risk of preeclampsia and obesity is an important risk factor for preeclampsia (Duckitt and Harrington, 2005). Moreover, we have demonstrated that insulin resistance together with adipocyte dysfunction might be involved in the pathophysiology of preeclampsia in obese women (Masuyama et al, 2010a, 2011).

The constitutive androstane receptor (CAR) is an orphan nuclear receptor and is highly expressed in the liver and small intestine, and at low levels in heart, skeletal muscle, brain, kidney and lung (di Masi et al., 2009). It was originally characterized as a nuclear receptor that can activate an empirical set of retinoic acid response elements without retinoic acid (Baes et al., 1994; Choi et al., 1997). CAR can be activated in response to xenochemical exposure, including phenobarbital-stimulated activation of a response element, NR1, found in the human and mouse *CYP2B* genes (Honkakoski et al., 1998; Sueyoshi et al., 1999). This phenobarbital response enhancer module is also located in the upstream region of the uridine diphosphate-5′-glucuronosyltransferase 1A1 (*UGT1A1*) gene and is activated by CAR (Masuyama et al., 2010b; Sugatani et al., 2001). In addition, activation of the bilirubin clearance pathway by CAR ligands is abolished in *CAR*-null mice (Huang et al., 2003), suggesting that the CAR–UGT1A1 pathway may play an important role in bilirubin clearance. Moreover, recent studies have linked CAR to lipid and glucose metabolism. The activation of CAR suppresses lipogenesis and lowers serum triglyceride levels by reducing the protein levels of the active form of sterol regulatory element-binding protein 1 (SREBP-1), a lipogenic transcription factor (Roth et al., 2008). The expression of the key hepatic gluconeogenic enzymes PEPCK and glucose-6-phosphatase (G6Pase) was also reported to be suppressed in phenobarbital-treated mice in a CAR-dependent manner (Ueda et al., 2002). Recent *in vivo* studies have demonstrated that the activation of CAR improves insulin sensitivity via glucose and lipid metabolic pathways including

Abbreviations: CAR, constitutive androstane receptor; CD, control diet; GTT, glucose tolerance test; G6Pase, glucose-6-phosphatase; HFD, high-fat diet; HOMA-IR, homeostasis model assessment-insulin resistance; ITT, insulin tolerance test; SCD-1, stearoyl-CoA desaturase 1; SREBP-1, sterol regulatory element-binding protein 1; TCPOBOP, 1,4-bis(2-(3,5-dichloropyridyloxy)) benzene.

* Corresponding author. Address: Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Department of Obstetrics and Gynecology, 2-5-1 Shikata, Kita-ku, Okayama 700-8558, Japan. Tel.: +81 86 235 7320; fax: +81 86 225 9570.

E-mail address: masuyama@cc.okayama-u.ac.jp (H. Masuyama).

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.07.047

PEPCK, G6Pase, SREBP-1 and stearoyl-CoA desaturase 1 (SCD-1), a key enzyme involved in the synthesis of unsaturated fatty acids and that CAR null mice showed spontaneous insulin insensitivity, which could be relieved by CAR ligand (Dong et al., 2009; Gao et al., 2009), suggesting that CAR plays some roles in insulin resistance.

CAR may play a role in insulin resistance, and obesity and insulin resistance appear to be involved in the pathogenesis of preeclampsia, thus we examined whether treatment with CAR ligands can ameliorate the signs of preeclampsia by improving insulin resistance and adipocyte function in high-fat diet (HFD)-induced obese pregnant mice. Because HFD has been demonstrated to worsen glucose metabolism during pregnancy (Liang et al., 2010), we employed HFD-induced obese pregnant mice. Also, we used TCPOBOP as a CAR ligand because TCPOBOP has been shown to bind specifically murine CAR and to activate CAR-mediated signaling (di Masi et al., 2009). Moreover, we examined whether TCPOBOP treatment affected the levels of other hormones including sex hormones and cortisol, which might be involved in insulin resistance (Barros et al., 2008; Livingstone and Collison, 2002; Ryan and Enns, 1988).

2. Materials and methods

2.1. Materials and animal procedures

1,4-Bis(2-(3,5-dichloropyridyloxy)) benzene (TCPOBOP), D-glucose and human insulin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Female, 8-week-old ICR mice were obtained from Charles River Co., Ltd. (Tokyo, Japan), and six female mice were examined per group for all *in vivo* experiments. After 4 weeks of feeding with the HFD (energy content: 62% fat, 18% protein and 20% carbohydrate) or a control diet (CD, 12% fat, 28% protein and 60% carbohydrate) purchased from Oriental yeast Co. (Tokyo, Japan), the mice were weighed and mated. Females were checked daily for postcopulatory plugs and the presence of a plug represented day 0.5 of pregnancy. Eight-week-old nonpregnant female mice as nonpregnant control and male mice for mating were fed with CD for four weeks before experiments or mating. The mice had free access to food and water, and their food consumption was estimated by weighing remaining food. Pregnant HFD- and CD-fed mice received once-weekly intraperitoneal injections of TCPOBOP (0.5 mg/kg) dissolved in corn oil or corn oil alone, which was started at day 0.5 of pregnancy. Twenty-week-old nonpregnant CD-fed female mice also received TCPOBOP treatment with same dose and interval of treatment. The systolic blood pressure of mice was measured at day 18.5 of pregnancy by the tail-cuff method using a Softron BP98A tail-cuff haemodynamometer (Softron, Tokyo, Japan) after the behavior and heart rate of the mice had stabilized. Blood pressure is reported as the mean of at least three measurements recorded during the same session, which had to vary by <5%. Most of the blood pressure values were within the required range once the mice had stabilized. The mice were transferred to metabolic cages at day 18.0 of pregnancy, and 12-h urine samples were collected. The urinary protein level was measured by the pyrogallol red method (Fujita et al., 1983). On day 18.5 of pregnancy, after the mice were anesthetized with ether, the fetal and placental weights were measured and the liver, white mesenteric adipose tissue and the placentas were removed, immediately frozen and stored at −70 °C until analysis. Total RNA was extracted using TRIzol reagent (Life Technologies Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. The mice were kept in a temperature- and light-controlled room with free access to food and water except during glucose (GTT) and insulin (ITT) tolerance tests. All animal procedures were approved by the Institutional Animal Care and Use Committee of Okayama University.

2.2. GTT, ITT and measurements of insulin, total triglyceride, adiponectin, leptin, estradiol, progesterone and cortisol levels

Mice at day 18.5 of pregnancy were fasted for 16 h before receiving an i.p. injection of D-glucose (2 g/kg body weight) for the GTT or for 4 h before receiving an i.p. injection of human insulin (1.0 U/kg body weight) for the ITT. Blood samples were taken before and at 30, 60, 90 and 120 min after the injection of glucose or insulin. Blood glucose levels were measured by the glucose oxidase method using a Medisafe automated analyser (Termo, Tokyo, Japan). Fasting insulin, total triglyceride, adiponectin and leptin levels were determined using ELISA kits (insulin and triglyceride: Morinaga Institute of Biological Sciences Inc., Yokohama, Japan; adiponectin and leptin: R&D Systems, Inc., Minneapolis, MN, USA; estradiol, progesterone and cortisol: Cayman Chemicals Co., Ann Arbor, MI, USA). Blood sample volumes for each measurement were 10–20 μl and total sample volume collected from each mouse was less than 200 μl, which was less than 5% of total blood volume. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as the fasting insulin concentration (μU/ml) × fasting glucose concentration (mg/dl)/405 (Hosker et al., 1985).

2.3. Real-time quantitative PCR

Real-time quantitative PCR was performed to measure the mRNA levels of *CAR*, *CYP2B10*, *PEPCK*, *G6Pase*, *SREBP-1*, *SCD-1*, *leptin* and *adiponectin* using a StepOne Real-time PCR System and a TaqMan RNA-to-CT Gene Kit (Applied Biosystems, Carlsbad, CA, USA). Specific primers for the mouse *CAR*, *CYP2B10*, *PEPCK*, *G6Pase*, *SREBP-1*, *SCD-1*, *leptin*, *adiponectin* and *β-actin* gene sequences were purchased from Applied Biosystems. Sequences of specific primers and accession numbers were described in previous reports (Maglich et al., 2002; Gao et al., 2009; van Schothorst et al, 2009). RNA samples (25 ng) were assayed in triplicate using 15 pmol of gene-specific primers and 5 pmol of gene-specific probes. Because we observed that there were no significant differences of *β-actin* expression under different diet conditions or TCPOBOP treatment using other housekeeping gene, GAPDH as a control (data not shown), mouse *β-actin* mRNA levels were measured as an internal control using a predeveloped TaqMan primer and a probe mixture (Applied Biosystems). The mRNA levels of the target genes were normalized by the *β-actin* mRNA levels.

2.4. Statistical analysis

Statistical analyses were performed by one-way ANOVA for comparison between non-pregnant mice and CD-fed pregnant mice, two-way ANOVA for comparison among 4 pregnant mice groups, HFD-fed or CD-fed with or without TCPOBOP treatment or among 4 groups, CD-fed nonpregnant and CD-fed pregnant mice with or without TCPOBOP treatment, and repeated measure ANOVA for GTT and ITT followed by Dunnett's test. Data are presented as means ± SD. Values of $p < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. CAR activation improves glucose tolerance and insulin sensitivity in HFD-fed obese pregnant mice

We performed GTTs and ITTs, measured serum insulin level and calculated HOMA-IR in non-pregnant mice, HFD-fed or CD-fed