Environmental Pollution xxx (2016) 1–13

Contents lists available at ScienceDirect

Environmental Pollution

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

Cadmium-induced immune abnormality is a key pathogenic event in human and rat models of preeclampsia☆

Qiong Zhang [a], Yinping Huang [b, *, 1], Keke Zhang [b], Yanjun Huang [b], Yan Yan [c], Fan Wang [a], Jie Wu [b], Xiao Wang [b], Zhangye Xu [a], Yongtao Chen [c], Xue Cheng [c], Yong Li [d], Jinyu Jiao [c], Duyun Ye [c]

[a] Department of Gynaecology and Obstetrics, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, 325000, Zhejiang, China
[b] Department of Gynaecology and Obstetrics, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, 325000, Zhejiang, China
[c] Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, Hubei, China
[d] Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, Hubei, China

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form
8 July 2016
Accepted 31 July 2016
Available online xxx

Keywords:
Preeclampsia
Cadmium
Angiotensin II type 1 receptor agonistic autoantibody
Activation-induced cytosine deaminase
Complement

ABSTRACT

With increased industrial development, cadmium is an increasingly important environmental pollutant. Studies have identified various adverse effects of cadmium on human beings. However, the relationships between cadmium pollution and the pathogenesis of preeclampsia remain elusive. The objective of this study is to explore the effects of cadmium on immune system among preeclamptic patients and rats. The results showed that the cadmium levels in the peripheral blood of preeclamptic patients were significantly higher than those observed in normal pregnancy. Based on it, a novel rat model of preeclampsia was established by the intraperitoneal administration of cadmium chloride (CdCl2) (0.125 mg of Cd/kg body weight) on gestational days 9–14. Key features of preeclampsia, including hypertension, proteinuria, placental abnormalities and small foetal size, appeared in pregnant rats after the administration of low-dose of CdCl2. Cadmium increased immunoglobulin production, mainly angiotensin II type 1-receptor-agonistic autoantibodies (AT1-AA), by increasing the expression of activation-induced cytosine deaminase (AID) in B cells. AID is critical for the maturation of antibody and autoantibody responses. In addition, angiotensin II type 1-receptor-agonistic autoantibody, which emerged recently as a potential pathogenic contributor to PE, was responsible for the deposition of complement component 5 (C5) in kidneys of pregnant rats via angiotensin II type 1 receptor (AT1R) activation. C5a is a fragment of C5 that is released during C5 activation. Selectively interfering with C5a signalling by a complement C5a receptor-specific antagonist significantly attenuated hypertension and proteinuria in Cd-injected pregnant rats. Our results suggest that cadmium induces immune abnormalities that may be a key pathogenic contributor to preeclampsia and provide new insights into treatment strategies of preeclampsia.

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that is characterized by hypertension, proteinuria and vascular abnormalities and often by intrauterine growth retardation (Roberts and Cooper, 2001) after the 20th week of gestation. It affects 2%–8% of pregnant women worldwide (Steegers et al., 2010), resulting in increased maternal and foetal morbidity and mortality. Numerous recent studies have shown that women with preeclampsia possess angiotensin II type 1-receptor-agonistic autoantibodies (AT1-AAs) those bind to and activate AT1 angiotensin receptors (AT1R) (Yang et al., 2015; Xia and Kellems, 2013; LaMarca et al., 2012; Sahay et al.,

Abbreviations list: PE, preeclampsia; AT1-AA, angiotensin II type 1receptor agonistic autoantibody; Cd, cadmium; complement component 5, C5; AID, activation-induced cytosine deaminase; CSR, class switch DNA recombination; SHM, somatichpermutation; SBP, Systolic blood pressure.

☆ This paper has been recommended for acceptance by David Carpenter.

* Corresponding author.
E-mail addresses: joan_zhang2002@sina.com (Q. Zhang), yphuangp@126.com (Y. Huang), zhangkeke0806@126.com (K. Zhang), wz5000@126.com (Y. Huang), 1019028268@qq.com (Y. Yan), 413258460@qq.com (F. Wang), wjjw307@163.com (J. Wu), 46506149@qq.com (X. Wang), 531953862@qq.com (Z. Xu), 51325023@qq.com (Y. Chen), 402719092@qq.com (X. Cheng), studying@hust.edu.cn (Y. Li), 402591446@qq.com (J. Jiao), yedy@mails.tjmu.edu.cn (D. Ye).
[1] Co-first author.

http://dx.doi.org/10.1016/j.envpol.2016.07.073
0269-7491/

2014; Brewer et al., 2013). The introduction of these autoantibodies into pregnant rats results in hypertension, proteinuria and a variety of other features of preeclampsia (LaMarca et al., 2009). These findings raise the intriguing possibility that preeclampsia may be a pregnancy-induced autoimmune condition that is characterized by the presence of disease-causing angiotensin-receptor-activating autoantibodies. However, the factors those contribute to the increased levels of AT1-AAs in preeclampsia and how these autoantibodies result in preeclampsia are still unclear.

With increased industrial development, cadmium (Cd) is an increasingly important environmental pollutant to both humans and animals (Thevenod and Lee, 2013; Kah et al., 2012). The population is exposed to Cd daily via the consumption of food and water polluted with this metal (Schlecht and Säumel, 2015; Cai et al., 2009), as well as by smoking (Al and Omu, 1999). Pregnant women are more vulnerable to Cd because of the greatly increased absorption and retention of Cd caused by nutritional deficiencies during pregnancy (Nishijo et al., 2004). Cadmium is a known endocrine disruptor (Knazicka et al., 2015) that affects the synthesis and/or regulation of several hormones. Studies have shown that cadmium has potent oestrogen-like activity in vivo (Nasiadek et al., 2011; Silva et al., 2013). According to several clinical and epidemiological studies, females can have stronger and more rapid immune responses than males upon antigen encounter (Kovats, 2015). This may occur because oestrogen can enhance immunoglobulin production by upregulation of the expression of activation-induced cytosine deaminase (AID) (Asaba et al., 2015), which is critical for the maturation of antibody and autoantibody responses. These results favour the hypothesis that Cd plays an important role in the immune system as well as oestrogen and it increases the production of autoantibodies that may induce pregnancy-specific hypertension.

A well regulated complement system is a prerequisite for a healthy pregnancy (Chow et al., 2009). The complement system can be initiated through the classical, lectin or alternative pathways. The activation of complement component 5 (C5) is a point of convergence for all three of the major complement activation pathways. The classical complement pathway is mainly initiated by antibody-dependent. Complement is activated by immune complexes of AT1-AA plus AT1R leading to generation of activation products C5a. C5a is a 74-amino acid fragment of C5 that is released during C5 activation, mediate the hypertension and functions as an anaphylatoxin that provokes a strong inflammatory response by activation of complement C5a receptors (C5aR) on multiple target cells, including inflammatory cells, endothelial cells, vascular smooth muscle cells, and epithelial cells (Rafail et al., 2015; Li et al., 2015; Shagdarsuren et al., 2010). Besides, a case report was published, where treatment with the C5 inhibitor eculizumab prolonged PE pregnancy by 17 days (Burwick and Feinberg, 2012). We found it reasonable to hypothesize that increased AT1-AA may contribute to C5 activation and an imbalance between C5a activation and regulation could be involved in PE.

Studies on the relationship of Cd and preeclampsia may provide new insights into the pathogenesis of preeclampsia and shed some light on getting new treatment strategies of preeclampsia.

2. Materials and methods

2.1. Patients

Patients at 28–40 weeks of gestation admitted to the First Affiliated Hospital of Wenzhou Medical University were identified by the obstetrics faculty. Severe PE was defined as either severe hypertension (systolic blood pressure $\geq$ 160 mmHg or diastolic blood pressure $\geq$ 110 mm Hg) or severe proteinuria (urinary protein excretion $\geq$ 5.0 g per 24 h). The blood pressure of all patients returned to normal levels and symptoms of proteinuria disappeared by 6 weeks postpartum. Patients with chronic hypertension, renal disease, collagen vascular disease, premature rupture of membrane and other complications of pregnancy were excluded from this study. Pregnant women with uncomplicated pregnancies were randomly selected to serve as controls. Preeclamptic patients diagnosed with severe disease (n = 20) and women with normotensive pregnancies (n = 20) were included in this study. The research protocol was approved by the Institutional Committee for the Protection of Human Subjects. Detailed information on the human subjects is presented in supplemental material.

2.2. Plasma analyses

Plasma samples from a cohort of preeclampsia patients (n = 20) and from women with normal pregnancies (n = 20) were used for biochemical assays. Plasma was prepared by centrifugation for 20 min at 4000 rpm at 4 °C. Cd in maternal blood and umbilical cord blood was measured by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500 with a Cd program). AT1-AA and C5a levels in plasma were measured with ELISA kits (YAJI Biological Technology Co. Ltd, Shanghai, China and USCN Business Co. Ltd, Wuhan, China) according to the manufacturer's instructions.

2.3. Animals

All animal studies were performed in Wistar rats purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). The animals were housed in a temperature-controlled room (23 °C) with a 12:12 light:dark cycle. All experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

2.3.1. Protocol 1 to confirm suitable timing and dosage of cadmium chloride to establish the PE model

Twenty-five pregnant rats were divided into five groups (normal pregnant control, D7 group, D9 group, D11 group and D13 group; n = 5 animals per group) according to a random number table. The rats were intraperitoneally injected with cadmium chloride (CdCl2; Sinopharm Chemical Reagent Co. Ltd, Mainland, China) at concentration of 0.25 mg of Cd/kg body weight (b.w.) daily for six days from gestational day (GD) 7–12 for the D7 group, from GD 9 to 14 for the D9 group, from GD 11 to 16 for the D11 group and from GD 13 to 18 for the D13 group. The other animals served as normal pregnant controls. The blood pressure of the rats in all groups was measured and compared to determine suitable injection times for the establishment of the preeclampsia model.

Another 20 pregnant rats were divided into four groups: a normal pregnancy group (n = 5), 0.0625 mg/kg Cd group (n = 5), 0.125 mg/kg Cd group (n = 5) and 0.25 mg/kg Cd group. The animals were intraperitoneally injected with sterile saline or CdCl2 at concentrations of 0.0625, 0.125 or 0.25 mg of Cd/kg b.w. at the suitable time as determined in the first step. Blood pressure, maternal body weight and foetal body weight were compared among the groups to determine the lowest effective dose.

2.3.2. Protocol 2 cadmium chloride administration to pregnant rats to induce a preeclampsia-like syndrome

Finally, 25 rats were randomly divided into a normal pregnant + Cd (NP + Cd) group (n = 5), a normal pregnant + normal saline (NP + NS) group (n = 5), a normal pregnant (NP) group (n = 5), a non-pregnant + (Non-p + Cd) group (n = 5) and a non-

Please cite this article in press as: Zhang, Q., et al., Cadmium-induced immune abnormality is a key pathogenic event in human and rat models of preeclampsia, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.07.073