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Expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and peroxiredoxin 6 (Prdx6) proteins in healthy and pathologic placentas of human and rat

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ABSTRACT

A relationship has been shown between preeclampsia (PE) and intrauterine growth restriction (IUGR) and oxidative stress (OS). Since such pregnancies experience OS, we aimed to detect the distribution pattern and expression levels of a transcription factor, Nuclear factor erythroid 2-related factor-2 (Nrf2) that has a role in the regulation of antioxidant enzymes, and peroxiredoxin 6 (Prdx6) an antioxidant enzyme, in human healthy, IUGR, PE and in groups of rat healthy and IUGR placentas using immunohistochemistry and Western blotting. Both Nrf2 and Prdx6 immunoreactivities were weaker in human and rat IUGR group placentas compared to human and rat control group placentas, respectively. Nrf2 and Prdx6 were immunostained in labyrinth trophoblasts, decidua, giant, glycogen and fetal vessel endothelial cells in rat control and IUGR group placentas. Nrf2 and Prdx6 immunoreactivities were seen in the decidua, syncytiotrophoblasts, villous stromal cells, and vascular endothelium in human control, IUGR and PE group placentas. Results of Nrf2 and Prdx6 Western blotting applied for rat and human placentas were compatible with the results of Nrf2 and Prdx6 immunohistochemical observations with regard to rat and human placentas. Down-regulation of Nrf2 and Prdx6 proteins in human and rat IUGR group placentas may have led to the formation of OS which may have impaired proliferation and invasion of cytotrophoblasts.

Introduction

The placenta is a regulator organ for many metabolic activities between mother and fetus. It is a critical organ affecting the outcome of pregnancy. Fetal growth is directly related to the placental development. Abnormal placental development, which is among the most important causes of early embryonic death, lies at the core of many common complications of pregnancy, such as preeclampsia (PE) and intrauterine growth restriction (IUGR) (Hemberger and Cross, 2001).

PE is a complex multisystem disorder and complicates 7–10% of all gestations (Tug et al., 2003). It presents serious risk of both maternal and fetal morbidity and mortality (Sibai et al., 2005). PE is characterized by high blood pressure and proteinuria in the second half of pregnancy (Uzan et al., 2011). Pre-term delivery, low birth weight, fetal death and neonatal death due to complications of pre-term delivery are common perinatal outcomes associated with PE (Ware-Jauregui et al., 1999). Fetal delivery is the only definitive treatment for threatening manifestations of symptoms. Whereas the pathophysiology of PE in particular remains incompletely understood, it appears to result from poor development of placental blood vessels (Redman and Sargent, 2005), which may disrupt the normal pattern of blood flow into the intervillous space, potentially risking ischemia/reperfusion injury (Burton et al., 2009). Indeed, there is general agreement that the induction of

Abbreviations: ARE, antioxidant-responsive element; Cys, cystein; Dexamethasone, dexamethasone 21-acetate; GPx, glutathione peroxidase; HO-1, hemeoxygenase 1; IUGR, intrauterine growth restriction; Keap1, Kelch-like ECH-associated protein 1; LOX-1, lectin-like oxLDL receptor-1; NAD(P)H, nicotinamide adenine dinucleotide phosphate; NQO1, NAD(P)H quinone oxido-reductase-1; Nrf2, nuclear factor erythroid 2-related factor 2; OS, oxidative stress; oxLDL, oxidized low-density lipoprotein; PBS, phosphate buffered saline; Prdx, peroxiredoxin; PE, preeclampsia; ROS, reactive oxygen species; RSA, recurrent spontaneous abortion; SOD, superoxide dismutase; γ-GCS, γ-glutamyl cysteine synthetase; 4-HNE, 4-hydroxynonenal.

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oxidative stress (OS) is a component of PE (Hung and Burton, 2006) supported by reports of increased pro-oxidant factors (Gandley et al., 2008; Many et al., 2000) and decreased anti-oxidants in the preeclamptic placenta (Many et al., 2000).

IUGR is defined as fetal weight below 10th percentile of a given population at the same gestational age (Roman et al., 2013; van Vliet et al., 2013). Vascular development of the placenta is inadequate in IUGR, resulting in impaired uteroplacental blood flow and poor nutrient and oxygen supply to the fetus (Aimot-Macron et al., 2013). Sustained hypoxemia and under-nutrition of the developing fetus can affect fetal programming of vital organs and an increased risk for disease later in life. IUGR due to placental insufficiency occurs in 5–10% of gestations and is a major determinant of perinatal morbidity and mortality (Neerhof et al., 2012; Aimot-Macron et al., 2013; Radulescu et al., 2013; van Vliet et al., 2013).

Although the architecture of the human and rodent placentas show minor differences, their overall structure and the molecular mechanisms of placental development are thought to be very similar. As a result, the rat placenta is increasingly used as a model to investigate the mechanisms of placental development (Rossant and Cross, 2001) and pregnancy-related problems in humans, such as those associated with diabetes, hypertension and IUGR (Vercruysse et al., 2006). Glucocorticoid induced IUGR is highly relevant because administration of synthetic glucocorticoids, principally dexamethasone, to women threatened by premature labor is widely used in clinical practice (Liggins and Howie, 1972; Trainer, 2002). Although glucocorticoids promote lung maturation, these actions are not without negative side effects. Exposure to glucocorticoids retards fetal growth in animal models and in humans (Benediktsson et al., 1993; Seckl, 1994; Gluckman, 2001; Sugden et al., 2001; McDonald et al., 2003) together with an increased risk of subsequent hypertension, cardiovascular disease and glucose intolerance in the adult offspring (Barker, 1997). The mode of action of dexamethasone in placental growth inhibition has still not been determined (Babat et al., 2005).

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a member of the CNC (cap' n' collar) family of regulatory proteins (Ikeda et al., 2004) and is a basic leucine zipper transcription factor that is essential for the regulation of antioxidant enzymes and the phase II detoxifying response (Perez-Leal et al., 2013). It plays an important role in inhibiting OS by up-regulating the Nrf2-regulated antioxidants hemeoxygenase 1 (HO-1), NAD(P)H: quinone oxidoreductase-1 (NQO1), γ-glutamylcysteine synthetase (γ-GCS) and Peroxiredoxin 6 (Prdx6) (Chowdhury et al., 2009; Wang et al., 2013). Under non-stress conditions, newly synthesized Nrf2 is captured by Kelch-like ECH-associated protein 1 (Keap1) and constitutively degraded through the cytoplasmic ubiquitin-proteasome pathway (Kobayashi et al., 2004). The presence of excess reactive oxygen species (ROS), nitric oxide, zinc and alkenals can modify cysteine residues on Keap1, thereby inactivating it (McMahon et al., 2010; Taguchi et al., 2012). Nrf2 is consequently stabilized and can translocate into the nucleus to promote the transcription of antioxidant genes via binding to the antioxidant response element (ARE) sequence (Kobayashi et al., 2004).

Prdx6 was initially discovered in yeast as a 25-kDa enzyme that protects against oxidative damage (Chae et al., 1994). Peroxiredoxins (Prdxs) are a widely distributed superfamily of peroxidases that use thiols to reduce hydrogen peroxide, a broad range of organic hydroperoxides, and peroxynitrite. There are six mammalian Prdx isoforms: 2-Cystein (Cys) Prdx group (Prdx1–4), atypical 2-Cys Prdx group (Prdx5), and 1-Cys Prdx group (Prdx6) (Kang et al., 1998; Rhee et al., 1999; Seo et al., 2000). Prdx1, 2 and 6 are localized particularly in the cytosol, Prdx 3 is expressed mainly in mitochondria, Prdx 4 is located in endoplasmic reticulum and also secreted extracellularly and Prdx5 is located in mitochondria and peroxisomes (Paula et al., 2013). Prdx6 is a sole 1-cys enzyme with both phospholipase A2 and peroxidase activities (Ahn et al., 2013) and is a unique antioxidant enzyme that can reduce phospholipid and other hydroperoxides (Chowdhury et al., 2009). Prdx6 is the only member of the 1-Cys Prdx group and catalyzes the reduction of phospholipid hydroperoxide (Manevich et al., 2002; Pak et al., 2002).

In this study we aimed to detect the immunolocalization and expression levels of Nrf2 and Prdx6 proteins in human healthy, PE and IUGR term placentas. We also intended to determine the immunolocalization and expression levels of Nrf2 and Prdx6 proteins in rat healthy and dexamethasone-induced IUGR group placentas to be able to conceive whether dexamethasone-induced intrauterine growth restricted rat placentas reflect similar results with human IUGR placentas and is a good model for studying intrauterine growth restriction mechanism.

Materials and methods

Animal tissues

Female Wistar rats weighing 250–300 g used for all experiments were maintained under standard conditions and exposed to 12/12 h light–dark cycles. After mating, the presence of sperm in the vaginal smear the following morning was designated as day 0 of pregnancy. Dexamethasone injection was performed based on some studies in the literature (Levitt et al., 1996; Sugden and Langdown, 2001). On day 13 of pregnancy, rats were subcutaneously injected with a dose of 100 μg dexamethasone 21-acetate (dexamethasone) (D1881; Sigma–Aldrich, St. Louis, MO, USA) in 0.1 ml 10% ethanol. The animals subsequently received daily injections of 200 μg/kg dexamethasone on days 14–19 of pregnancy. Control animals were injected with 10% ethanol subcutaneously on corresponding days of pregnancy. Eight 10% ethanol injected (control group) and eight dexamethasone injected (IUGR group) female Wistar rats were sacrificed on day 20 of pregnancy and placentas were obtained. The experimental protocols were approved by the Animal Care and Usage Committee of Akdeniz University and were in accordance with the guidelines of the International Association for the Study of Pain.

Human tissues

Term placentas from healthy women were obtained immediately after cesarean section and were used as control group ($n=6$). Control women had no history of pregnancies with PE, recurrent spontaneous abortion (RSA) or IUGR. Placentas from women with PE ($n=6$) and IUGR ($n=6$) were obtained immediately after cesarean deliveries. The gestational age of control group placentas ranged from 36 to 40 weeks. The gestational age of PE and IUGR specimens ranged from 35 to 40 weeks. The samples were supplied from the Department of Obstetrics and Gynecology at Akdeniz University, Medical Faculty after informed consent of patients. The Ethical Committees of Medical Faculty of Akdeniz University approved the consent forms and protocols. Pre-eclamptic cases were defined as persistent blood pressure above 140/90 mmHg and proteinuria of more than 0.3 g/24 h or 2+ or higher according to a dipstick test, developing after 20 weeks of pregnancy. IUGR was defined as birth weight below the 10th centile of customized birth weight for gestational age (van Vliet et al., 2013). Exclusion criteria of the study were pre-existing hypertension treated with antihypertensive drugs, diabetes mellitus, gestational diabetes, renal disease, heart