

New Insights into the Pathogenesis of Preeclampsia – The Role of Nrf2 Activators and their Potential Therapeutic Impact

Neue Einblicke in der Pathogenese der Präeklampsie – die Rolle von Nrf2-Aktivatoren und ihre potenzielle therapeutische Wirkung

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Abstract



Preeclampsia (PE), characterized by proteinuric hypertension and occurring in 2–3% of all pregnancies, is one of the leading causes of maternal, fetal and neonatal morbidity and mortality. The etiology of PE still remains unclear and current treatments for this devastating disorder are still limited to symptomatic therapies. Placental oxidative stress may be a key intermediate step in the pathogenesis of PE; it has been related to excessive secretion of multiple antiangiogenic factors, mainly soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng). The nuclear factor-erythroid 2-like 2 (Nrf2) pathway is one of the most important systems that enhance cellular protection against oxidative stress. Nrf2 serves as a master transcriptional regulator of the basal and inducible expression of a multitude of genes encoding detoxification enzymes and antioxidative proteins. Evidence for a link between Nrf2 and restoring the balance between pro- and antiangiogenic factors mainly through its downstream target protein heme oxygenase-1 (HO-1) has lately been discussed. HO-1 metabolizes heme to biliverdin, iron and carbon monoxide (CO). CO enhances vascular endothelial growth factor (VEGF) synthesis in vascular smooth muscle and promotes its relaxation and hence vasodilatation. In addition, HO-1 has been shown in vitro to inhibit the production of sFlt-1. A recent animal study demonstrated that the induction of HO-1 in a mouse model of PE attenuates the induced hypertension in pregnant mice. This provides compelling evidence for the protective role of Nrf2/HO-1 in pregnancy and identifies this pathway as a target to treat women with PE. We summarize the recent findings on the involvement of Nrf2 in the pathogenesis of PE, and provide an overview of the possible beneficial effects of Nrf2 inducers in PE.

Zusammenfassung



Präeklampsie (PE) ist charakterisiert durch Gestationshypertonie und Proteinurie; mit einer Prävalenz von 2 bis 3% zählt sie zu den häufigsten Schwangerschaftserkrankungen. Präeklampsie gehört zu den führenden Ursachen für fetale, neonatale und mütterliche Morbidität und Mortalität. Die Ätiologie dieses Syndroms ist noch unbekannt, und die Behandlung richtet sich daher nach den Symptomen. Studien haben gezeigt, dass oxidativer Stress in der Plazenta eine wichtige Rolle bei der Pathogenese der Präeklampsie spielen könnte. Plazentaler oxidativer Stress könnte zu einer erhöhten Freisetzung von antiangiogenetischen Faktoren wie fms-like Tyrosine Kinase-1 (sFlt-1) und Soluble Endoglin (sEng) führen. Der Nuclear-Factor-Erythroid-like-2-(Nrf2-)Signalweg ist ein wichtiges System, das die Zellen vor oxidativen Stress schützt. Die Expression vieler antioxidativer Enzyme wird von Nrf2 verstärkt. Es wurde ferner berichtet, dass das Nrf2-System eine Rolle bei der Regulierung der Balance von pro- und antiangiogenen Faktoren spielt, indem es das Zielprotein Hämoxxygenase-1 (HO-1) hochreguliert. HO-1 katalysiert die Oxidation von Häm zu Biliverdin, Eisen und Kohlenmonoxide (CO). CO stimuliert die Proteinsynthese des vaskulären endothelialen Wachstumsfaktors VEGF in vaskulären glatten Muskelzellen, und kann auch die Relaxation der glatten Muskulatur (Gefäßerweiterung) induzieren. Zudem kann HO-1 in vitro die Freisetzung von sFlt-1 hemmen. Darüber hinaus wurde kürzlich in Studien gezeigt, dass die Aktivierung von HO-1 in verschiedenen Tiermodellen für Präeklampsie zu einer Blutdrucksenkung führt. Diese Studien zeigen, dass der Nrf2/HO-1 Signalweg eine präventive Rolle bei Präeklampsie spielen könnte. Ziel dieser Übersichtsarbeit ist es, die neuesten wissenschaftlichen Studien zur Beteiligung von Nrf2 an der Pathogenese von Präeklampsie zu-

Introduction

Hypertensive disorders of pregnancy complicate 5–10% of pregnancies and can lead to serious maternal illness or death. Preeclampsia (PE), the most serious of these disorders, is the second leading cause of maternal death worldwide, and results in 63 000–72 000 maternal deaths each year. Over 99% of these deaths occur in low- and middle-income countries [1]. Although PE complicates 2–3% of pregnancies in UK, PE is still the second leading cause of maternal death with an incidence of about 0.83 deaths per 100 000 maternities in UK for 2006–2008 [2].

According to the criteria of the American College of Obstetricians and Gynecologists (ACOG), PE is characterized clinically by a new onset of hypertension ($\geq 140/90$ mmHg) and proteinuria (≥ 300 mg/day) after 20 gestational weeks [3]. Advanced-stage clinical symptoms include seizures, renal failure, intrauterine growth restriction (IUGR), and/or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome.

PE has an impact on the health of both the mother and the fetus, and has also a long-term impact on health. Systemic reviews and meta-analysis showed that women with PE are twice as likely to develop cardiovascular disease later in life [4, 5]. Although the estimated 10-year cardiovascular disease risk is low (less than 5%) after delivery, cardiovascular disease risk is expected to increase rapidly with increasing age [6].

PE has been termed the “disease of theories”, reflecting the confusion about the etiology and pathophysiology of PE [7]. Nevertheless, it appears likely that the placenta releases substances that cause endothelial dysfunction in the maternal blood vessels of susceptible women. It involves generalized damage to the maternal endothelium in the kidneys, liver, and blood vessels, leading to blood pressure elevation, the most conspicuous sign of the disease [8].

Placental oxidative stress is considered to promote the release of the aforementioned factors that may be involved in endothelial cell dysfunction. The principal pathology appears to be insufficient uteroplacental blood supply [9], which mainly results from reduced trophoblast invasion.

The shallow trophoblast invasion in PE results in defective spiral artery remodeling followed by high-resistance vessels and reduced placental perfusion [10]. The consequence is retention of vasoreactivity in the myometrial segments of these arteries, leading to intermittent perfusion of the intervillous space and hence to fluctuating oxygen concentrations that results in oxidative stress within the placenta [11].

To counteract oxidative stress, the placenta produces several antioxidants including heme oxygenases (HO-1, HO-2), copper zinc superoxide dismutase (SOD), and glutathione peroxidase (GPx) [12].

Exposure to reactive oxygen species switches on a battery of genes encoding antioxidant enzymes. This coordinated response is regulated via the Antioxidant Response Element (ARE) contained within the promoter regions of the so-called “safeguard” genes [13, 14]. Activation of the nuclear factor-erythroid 2-like 2 (Nrf2) as a consequence of oxidative stress or vascular endothelial growth factor (VEGF) initiates and enhances the transcription

sammenzufassen. Zudem möchten wir einen Überblick über das therapeutische Potenzial von Nrf2-Aktivatoren bei der Präeklampsie geben.

of these “safeguarding” genes, thus protecting the cells against oxidative stress as well as a wide range of other toxins [15].

Wruck et al. (2009) presented the first experimental data showing that Nrf2 is exclusively active within the cytotrophoblast of the preeclamptic placenta, strongly suggesting that these cells suffer from oxidative stress caused by excess production of the reactive oxygen species (ROS) [16]. Moreover, induction of Nrf2 increases the protein levels of VEGF via its target protein HO-1 and its metabolite carbon monoxide (CO) [17].

Because of Nrf2’s ability to induce protein synthesis of VEGF, the impairment of Nrf2 signaling in the extravillous trophoblast (EVT) of preeclamptic women has been recently suggested as limiting the ability for invasion of these cells [18].

The present review aims to highlight recent research progress in the field of the transcription factor Nrf2 with regard to its role in the development of PE and its potential use as a therapeutic target.

Oxidative Stress in PE

In normal pregnancies, the production of ROS and lipid peroxidation increases toward the end of pregnancy [19], whereas antioxidant capacity increases in order to maintain oxidative balance throughout pregnancy [19]. In addition, a normal pregnancy enhances the general inflammatory response, especially toward the end of the third trimester [20]. This includes the activation of monocytes, granulocytes and lymphocytes during the third trimester, all of which produce ROS [21].

Oxidative stress has been implicated in promoting PE. The placental stresses of PE mostly develop in early onset disease and stimulate the release of circulating factors that cause the maternal syndromes [20]. It has been shown that the main manifestations of this syndrome – represented by hypertension and proteinuria – are secondary to diffuse endothelial dysfunction [22]. Oxidative stress occurs when the production of reactive oxygen species overwhelms the intrinsic antioxidant defenses. It may induce a wide range of cellular responses, depending on the severity of the episode and the compartment in which ROS are generated [20]. Indisputable evidence of placental oxidative stress in PE, including increased concentrations of protein carbonyls, lipid peroxides, nitrotyrosine residues, and DNA oxidation [21], confirm this theory.

What causes this oxidative stress? It is thought to be vascular, because early onset PE is associated with a weak invasion of the EVT and consequently deficient remodeling of the spiral arteries [23, 24]. In normal pregnancies, the remodeling of the uterine and placental vessels generates free radicals, which are normally controlled by appropriate antioxidant levels. In PE, it has been observed that ROS are increased, and the levels of several detoxifying enzymes are reduced [25]. Stepan et al. (2004) proved a correlation between impaired uteroplacental perfusion and decreased plasma antioxidant capacity. They showed that second-trimester pregnancies with pathological uterine perfusion are characterized by decreased total antioxidant capacity in the maternal circulation [26]. Their results added strength to the two-stage hypothesis whereby decreased plasma antioxidant capacity

as a result of reduced placental perfusion only reflects stage one and additional factors such as maternal genetic susceptibility or dyslipidemia are required to promote the later disease [27]. In addition, because of the high contractility of the spiral artery of the myometrial segment, it is hypothesized that the ensuing high-pressure flow causes hydrostatic damage to placental villi and perfusion due to intermittent pulses of fully oxygenated arterial blood; the latter is presumed to lead to the fluctuations in oxygen delivery that predispose to oxidative stress [20]. According to Redman (2011), the placental problem, at least in the early stages, is due neither to hypoxia nor to reduced flow but rather to oxidative stress and physical disruption of placental villous architecture [20]. This hypothesis is supported by *in vitro* experiments; Hung et al. (2001) indicated that hypoxia/reoxygenation is a potent inducer of oxidative stress in term placental explants, much more than hypoxia alone [28].

Nrf2/ARE System

Nrf2 is a member of the cap'n'collar transcription factor family with a conserved basic region-leucine zipper domain that binds to the Antioxidant Response Element (ARE), a regulatory element in promoter regions of several genes encoding phase II detoxification enzymes (e.g. glutathione S transferases) and antioxidant proteins (e.g. NADPH quinone oxidoreductase – NQO1 and HO) [15].

Under normal conditions, Nrf2 is bound to Keap1, resulting in lowered levels in the cells because of rapid proteasomal degradation. Keap1 acts as a negative regulator of Nrf2 and as a sensor of oxidative and/or electrophilic stress. Once reactive thiols of Keap1 are modified by electrophiles or ROS, ubiquitination of Nrf2 is readily inhibited, thereby rescuing newly synthesized Nrf2 from proteasomal degradation and allowing for its accumulation in the nucleus [29] (● Fig. 1).

Nrf2 mediates a broad-based set of adaptive responses to intrinsic and extrinsic cellular stresses [29]. Several studies have reported that this factor exerts cellular protection against a wide variety of toxic insults (carcinogens, electrophiles, ROS, inflammation, calcium disturbance, ultraviolet light, and cigarette smoke) [30]. Our study group recently showed that VEGF has the ability to activate the Nrf2 system without intracellular ROS production [17]. Results from animal models highlighted the impact of this factor in several pathological conditions: hemolytic anemia, smoke-induced emphysema, and neurodegenerative disorders (traumatic brain injury, ALS, and Alzheimer's) [30, 31]. More recently, the involvement of this factor in the pathogenesis of PE has been discussed [16–18, 32, 33].

The antioxidative effects of Nrf2 in different biological and pathological conditions are well accepted. However, Nrf2 is still under intensive research because of the growing evidence that the Keap1/Nrf2 pathway also cross-talks with other molecular pathways and transcription factors [29].

Nrf2 in Preeclampsia

Wruck et al. (2009) [16] provided the first experimental data that Nrf2 is active exclusively within cytotrophoblasts of preeclamptic placentas, strongly suggesting that these cells suffer from oxidative stress caused by excessive production of ROS.

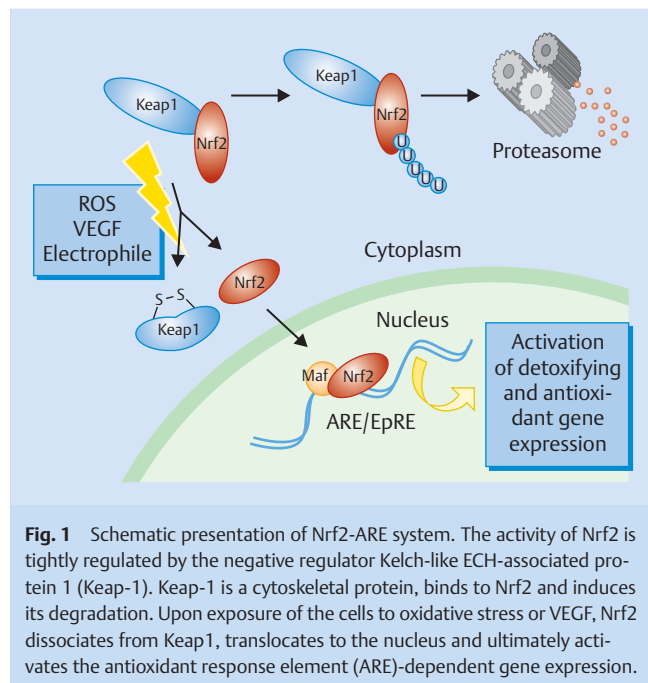


Fig. 1 Schematic presentation of Nrf2-ARE system. The activity of Nrf2 is tightly regulated by the negative regulator Kelch-like ECH-associated protein 1 (Keap-1). Keap-1 is a cytoskeletal protein, binds to Nrf2 and induces its degradation. Upon exposure of the cells to oxidative stress or VEGF, Nrf2 dissociates from Keap1, translocates to the nucleus and ultimately activates the antioxidant response element (ARE)-dependent gene expression.

Cell models have also served as a useful tool to study Nrf2 properties against cytotoxicity. Nrf2 has been studied in cell models related to pregnancy and placental development, such as human umbilical vein endothelial cells (HUVECs) [34] and BeWo cells (a model for syncytiotrophoblast formation [17]). The activation of HO-1 by Nrf2 protected cells against oxidative damage. Interestingly, the Nrf2 inducer used in this study has been shown to exert a positive effect on the activation of VEGF, and the disruption of Nrf2 signaling impairs the angiogenic capacity of trophoblasts [17] and endothelial cells [34].

Loiset et al. (2012) analyzed the single genes, canonical pathways and gene-gene networks that are likely to play an important role in the pathogenesis of PE. They reported that the Nrf2-mediated oxidative stress response was overrepresented in the decidua of patients with PE [35], consistent with the results from the first study of Wruck et al. (2009) on preeclamptic placental villi. On the other hand, a recent study demonstrated that placentas from preeclamptic patients showed a reduced Nrf2 activation associated with decreased HO-1 mRNA [32]. These authors provided further evidence for the disruption of Nrf2 signaling, which failed to increase the antioxidative genes in the placenta. As a consequence, both mother and fetus may be affected by oxidative stress.

Genetic profiling of highly migratory EVT and villous cytotrophoblast revealed that lower HO-1 expression is associated with lower cell motility and trophoblast invasion [36]. The invaded trophoblast of early onset IUGR and preeclampsia has been described as having impaired Nrf2 signaling compared to the control, as seen in immunohistochemistry [18]. Collectively, so far there have been too few studies that have clarified the role of Nrf2 in pregnancy-related disorders. But although the findings have been mixed and the efficacy noted can be difficult to corroborate, the current few studies that have been able to draw any structured conclusions agree that the Nrf2 signaling is disturbed in placentas from patients with PE, indicating without a doubt the role of Nrf2 in the pathogenesis of PE.

Potential Therapeutic Effects of Nrf2 Inducers in PE

Heme oxygenase-1

HO-1 is an antioxidant enzyme that catalyzes the rate-limiting step in the oxidative degradation of heme into equimolar amounts of biliverdin, carbon monoxide (CO), and free iron (Fe²⁺) [37, 38]. Biliverdin is then converted by biliverdin reductase into bilirubin. These two metabolites of heme breakdown, CO and bilirubin, have important functions that give HO-1 its vasodilatory, anti-inflammatory, antiapoptotic, antioxidant, and cytoprotective properties [39]. The promoter of the HO-1 gene contains consensus binding sites for multiple transcription factors including Nrf2, AP-1, AP-2, NFκB, HNF-1 and others, and one of the most crucial ones appears to be Nrf2 [15].

It was shown that HO-1 induction or CO administration in HUVECs inhibits their release of sFlt-1 and sEng [40]. Both of these antiangiogenic factors contribute to endothelial dysfunction in PE [8, 41–44], suggesting that HO-1 exerts a proangiogenic phenotype. HO-1 is also able to offer protection against sFlt-1 while bypassing this effect [39, 40]. In a sFlt-1-hypertensive rat model the activation of HO-1 reduced blood pressure [45]. Moreover, the inhibition of HO-1 increased blood pressure in pregnant rats [45].

HO-1 induces VEGF in endothelial cells, keratinocytes, macrophages and tumor cells, and the delayed process of wound healing in HO-1 knockout mice confirmed the proangiogenic effects of HO-1 [46]. Studies of HO-1 knockout mice showed also that a partial deficiency in HO-1 is associated with morphological changes in the placenta and elevations in maternal diastolic blood pressure and plasma sFlt-1 levels [47]. The expression of HO-1 can be induced by many compounds, some of which have therapeutic properties such as statins [48], probucol and probucol analogues [49], natural Nrf2 activators (kavalactones methysticin [50], sulforaphane [51], andrographolide [52]), and others. These findings may justify the hypothesis that pharmacological interventions to increase the activity of HO-1 in the placenta are useful options to treat PE by restoring disturbed maternal cardiovascular functions.

Unfortunately, statins have been identified as teratogenic and the risks associated with probucol are not well studied. Natural Nrf2 inducers may therefore be a promising future option to treat mothers with PE.

Hypotensive Effects of Nrf2 Activation

Two randomized placebo-controlled trials enrolling nulliparous women investigated the effects of antioxidant vitamin supplementation on the development of PE. Supplementation with vitamin C and E was found to have no significant effect on the risk of developing PE, while more low birthweight babies were born to women who took the high-dose antioxidants [53, 54]. Elsen et al. (2012) reported a significant difference in the levels of the antioxidative vitamins E and A between patients with severe PE and those with a mild form of the disease [55], suggesting that dietary supplementation of the antioxidants may not be as helpful as the endogenous induction of these enzymes and that this effect can be achieved through the activation of Nrf2.

Several Nrf2 downstream target genes, including NADPH oxidase, superoxide dismutase, thioredoxin and HO-1, have been shown to protect against hypertension associated with different disorders [45, 56]. In an animal model of deoxycorticosterone

acetate (DOCA)-salt-induced hypertension, activation of Nrf2 via epicatechin prevented both an increase in systolic blood pressure and proteinuria induced by DOCA-salt treatment [57]. Interestingly, a randomized clinical trial of the effect of bardoxolone methyl, an agent that protects cells from radiation-induced damage through Nrf2-dependent pathways [58], in patients with chronic kidney disease and type 2 diabetes mellitus showed that treatment with this agent improved renal function with blood pressure remaining constant in these patients [59]. Furthermore, in a recent study by Vanhees et al. (2013) the authors showed that in utero exposure to flavonoids, Nrf2 inducers, not only attenuated DNA damage induced by oxidative stress in the liver of mice embryos but also had long-lasting effects and triggered the antioxidant system in adult mice [60]. These are still new perspectives for the rational design and development of a novel class of Nrf2 activators to treat pregnancy-related disorders, and this will need much more investigation.

Conclusion

The role of Nrf2 in pregnancy complications is a new finding in research. It has become obvious that Nrf2 plays a key role in many pathways such as trophoblast invasion, preserving endothelial function and restoring the balance between pro- and antiangiogenic factors. Clinical trials with antioxidant interventions in preeclampsia have not proved successful. Based on the current literature, enhancement of the endogenous protection system against oxidative stress by Nrf2 activation seems to be a promising alternative. Thus, supplementation with Nrf2 inducers in a therapeutic design could be an innovative target which deserves further intensive research in well-conducted studies.

Conflict of Interest

None.

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