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Oxidative stress in pre-eclampsia; have we been looking in the wrong place?

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Introduction

Pre-eclampsia is a disorder of late pregnancy. It is a major cause of maternal and perinatal morbidity and mortality, accounting for nearly 18% of all maternal deaths worldwide; an estimated 77,000 maternal deaths per year (1). Poor placentation is considered to be an initial cause of the placental ischemia (2). Placental ischemia in turn gives rise to oxidative stress in the placenta and leads to shedding of syncytiotrophoblast debris into the maternal circulation provoking a systemic maternal inflammatory response and release of sFLT and sENG causing maternal vascular endothelial dysfunction. The ubiquitous nature of the maternal vascular endothelium accounts for the diverse multi-system nature of pre-eclampsia.

Currently there is no treatment for pre-eclampsia except delivery of the placenta and the baby, with the attendant risk of iatrogenic prematurity and significant neonatal morbidity and mortality. As a result, intensive research endeavours have focused on defining the molecular mechanisms of pre-eclampsia and the identification of new pre-symptomatic biomarkers of the condition. This review focuses on the role of elevated oxidative stress in the pathology of pre-eclampsia and potential therapeutic agents targeting oxidative stress that may prevent or ameliorate this disorder.

Reactive oxygen species (ROS)

Reactive oxygen species are typically recognised as two groups; free radicals and non-radical products. Free radicals include superoxide (O^{2-}), the hydroxyl radical (HO), lipid peroxy-radicals (LOO) and alkoxy-radicals (LO). Non-radical derivatives are more stable with a longer half-life, however, they can have strong oxidant properties. Non-radicals include hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hypochlorous acid ($HOCl^-$) (3). O^{2-} acts as an oxidant as well as a reductant in biological systems (4).

O^{2-}serves as an origin for many other biologically relevant ROS including H_2O_2, HO radical and $ONOO^-$ (3). H_2O_2 is created by dis-mutation of O^{2-}, this molecule can occur spontaneously or it can be promoted by superoxide dismutase (SOD). Hydroxyl radical (HO) is created when O_2 donates one electron to H_2O_2 in a reaction known as the Haber Weiss Reaction. The HO molecule is a highly reactive oxidant that attacks a variety of biomolecules such as lipids, proteins and DNA. Peroxynitrite ($OONO^-$) results from the spontaneous reaction between O_2 and NO. $OONO^-$ is known to be a very strong oxidant and it can react with lipids, DNA and proteins (3). This molecule reacts and modifies proteins and other cellular structures inflicting oxidative damage on these molecules.

Sources of ROS

Sources of ROS include NADPH Oxidase, xanthine oxidase and the mitochondria. NADPH oxidases are activated by a variety of physiological and pathophysiological stimuli including inflammatory cytokines, mechanical forces and growth factors. Xanthine oxidoreductase exists in two forms; xanthine oxidase and xanthine dehydrogenase. Xanthine oxidase transfers electrons to oxygen from NADH and uric acid and generates O_2^- and H_2O_2, whereas xanthine dehydrogenase transfers the electrons from hypoxanthine and xanthine to NAD^+ generating NADH and uric acid (3). When a critical cysteine residue is oxidised by peroxynitrite, xanthine dehydrogenase (XDH) is converted to xanthine oxidase. Xanthine oxidase has been shown to contribute to experimental hypertension in animal models, however currently there is limited evidence supporting the role of xanthine oxidase in human hypertension (5). Mitochondria are responsible for ATP production in the cell through oxidative phosphorylation. The electron transport chain involves NADH and flavinadenine dinucleotide (FADH) which act as electron transporters. Electrons go through four stages and finally electrons are transferred to an oxygen molecule. During normal mitochondrial function, electrons transfer from one complex to the next efficiently and there is minimal loss or leakage from the electron transport chain. However, oxidative stress is inherently linked to mitochondrial dysfunction due to disruption in the electron transfer between acceptors during oxidative phosphorylation in the inner mitochondrial membrane resulting in electron leakage.

The natural defence against ROS comprises both enzymatic and non-enzymatic systems. Superoxide dismutase (SOD) is a well-known antioxidant enzyme that prevents free radical mediated injury *in vivo* and *in vitro* by metabolising superoxide anions that are known to damage human tissues (6). SOD is categorised into three isoforms, cytosolic CU, Zn-SOD, and mitochondrial Mn-SOD, which catalyse the dismutation of O_2^- into H_2O_2 and O_2. A decrease in the endogenous SOD antioxidant defence systems weakens the normal metabolic removal of O_2. Additionally, endogenous protein alpha-1-microglobulin acts as a radical scavenger and an antioxidant. It provides protection to tissue and protects the mitochondria from oxidative damage. Non-enzymatic antioxidants act as ROS scavengers and include vitamins A, C and E; glutathione, bilirubin and uric acid. Vitamin E is a fat-soluble vitamin and its main role is to protect against lipid peroxidation. Vitamin C scavenges free radicals in aqueous solution.

Oxidative stress and pre-eclampsia

Oxidative stress is an imbalance between the production of ROS and antioxidant defences (3), resulting in increased levels of ROS with resultant damage of cellular components including DNA, proteins and lipids. Normal pregnancy is characterised by a low grade oxidative stress; there are increased circulating levels of oxidised low-density lipoproteins and a reduction in total antioxidant

capacity in pregnant women when compared with non-pregnant women (7). Excessive oxidative stress is generally thought to be involved in the pathology of many pregnancy-related disorders such as fetal growth restriction (FGR), pre-eclampsia and miscarriage. Dysfunctional placentation is proposed to provoke a hypoxic reperfusion injury causing elevated oxidative stress in pre-eclampsia. By 10–12 weeks' gestation in normal pregnancy maternal blood flow in the placenta causes a local increase in oxygen and elevation in the activity of the antioxidant enzymes (8). However, in pregnancies subsequently complicated by pre-eclampsia there is a decrease in antioxidant enzyme activity at the same gestation (9).

ROS are generally unstable and have a very short half-life, therefore accurately assessing O_2^- and H_2O_2 in the clinical setting is difficult. To address this, methods have been developed to measure stable markers of ROS that reflect an oxidative stress status. Biomarkers of oxidative stress in human samples include serum lipid hydroperoxides, plasma malondialdehyde (MDA) or urine F2-isoprostanes and uric acid (10). Lipid peroxidation involves polyunsaturated fatty acids, including phospholipids, glycolipids and cholesterol that are vulnerable targets of oxidation. Increased lipid peroxidation plays a vital role in the pathology of pre-eclampsia by provoking endothelial dysfunction. MDA levels have been found to be significantly higher in pre-eclampsia in comparison with uncomplicated pregnancy (7). Additionally, antioxidant SOD levels were significantly lower in pre-eclampsia in this study (7). F2-isoprostanes are prostaglandin-like compounds produced *in vivo* by free radical induced peroxidation and are measured in human tissues and biological fluids (11). In women with pre-eclampsia, isoprostanes have been shown to be elevated in the placenta (12). Uric acid is the end product of purine degradation catalysed by the enzyme xanthine dehydrogense and xanthine oxidase and its production is increased in oxidative stress (13). Hyperuricemia in pre-eclampsia was thought to result exclusively from reduced renal clearance, however levels of uric acid are now also believed to increase by trophoblast breakdown, cytokine release and ischemia.

In recent years, overproduction of cell-free fetal haemoglobin (HbF) has been implicated as a new pathological factor of pre-eclampsia (14). Haemoglobin reacts spontaneously with oxygen generating free oxygen radicals. Haemoglobin and its degradation products are toxic and can lead to oxidative stress in the maternal circulation (15). A recent study of 433 women in early pregnancy, 86 of whom developed pre-eclampsia measured serum HbF levels from the first trimester and reported that the mean concentration of HbF in the women who went on to develop pre-eclampsia was significantly higher than in the control group (16). Research from our group demonstrated elevated levels of several haemoglobin related metabolites including heme, bilirubin and biliverdin which were shown to be all increased in pre-eclampsia patients (17). The metabolite heme binds to nitric oxide causing vasoconstriction, which may be central in pre-eclampsia.

Targeting Oxidative stress in pre-eclampsia

Vitamins

Vitamin C and E are exogenous antioxidants known to down-regulate NADPH oxidase, a major source of ROS in the vascular wall, and also up regulate eNOS, which leads to vasorelaxation and a reduction in blood pressure (18). The therapeutic potential of these particular antioxidants in the prevention of conditions associated with oxidative stress is supported by an extensive evidence base comprising of experimental studies (19), observational studies (20) and small clinical studies (21). However, in stark contrast, large scale appropriately powered randomised clinical trials (RCTs) have been

overwhelmingly disappointing. The VIP trial was a RCT of vitamin C and vitamin E in 2410 women identified as being at an increased risk of pre-eclampsia (22). This study assigned the women with 1000mg vitamin C and 400 IU vitamin E or the matched placebo daily from the period of the second trimester of pregnancy until delivery. This trial concluded that supplementation with vitamin C and vitamin E did not prevent pre-eclampsia. However, a secondary outcome from this clinical trial showed that vitamin supplementation increased the rate of babies born with low birthweight (22). Another multicentre, randomised, double-blinded trial was carried out on 10,154 nulliparous women looking at vitamin C and vitamin E to prevent complications in pregnancy (23). The primary outcome for this clinical trial was severe pregnancy-associated hypertension or severe or mild hypertension with many clinical outcomes such as eclamptic seizures. From their data in this clinical trial, supplementation with vitamin C and E did not reduce the incidence of pregnancy-associated hypertension or pre-eclampsia in low risk nulliparous women (23). In comparison with the VIP trial, this study did not find significance between group differences in the rates of low birth rate and stillbirth. DAPIT was a randomised placebo-controlled clinical trial in the UK and Northern Ireland from 2003-2008 assessing vitamin C and vitamin E supplementation for the prevention of pre-eclampsia in women with type 1 diabetes (24). Women in this study were randomly allocated to receive 1000 mg of vitamin C and 400 IU vitamin E or matched placebo every-day from between 8 weeks' gestation and 22 weeks gestation until delivery. This study showed no significant differences between vitamins and the placebo groups for any of the maternal outcomes. However, plasma ascorbate concentrations for both vitamins were significantly higher in the women that took the vitamins in comparison to the placebo group at 26 weeks' gestation and 34 weeks' gestation with a low antioxidant status at baseline (24).In summary, in contrast to a significant supportive pre-clinical research base, these large clinical trials suggest that antioxidant therapy is ineffective in the treatment of disorders such as pre-eclampsia.

Nitric Oxide

Nitric oxide (NO) is a major endothelium-derived vasoactive mediator (25) which acts as a potent vasodilator regulating vascular tone and tissue blood flow, as well as inhibiting platelet aggregation and leucocyte adhesions on the endothelial surface (26). NO is synthesised by NO synthase (NOS) and activates soluble guanylyl cyclase to convert guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which is then degraded by phosphodiesterase 5 (PDE5) to guanosine monophosphate (GMP). Interruption of both NO and its secondary messenger cGMP has been implicated in the pathophysiology of pre-eclampsia (27). NO signalling cascade is important for the placental function and development of vascular network. The altered balance of NO and ROS are thought to play a critical role in the pathogenesis of pre-eclampsia (28). NO causes dilation of the utero-placental arteries, which is essential for trophoblast invasion and remodelling of the endothelium (29). From these findings, it was hypothesised that low levels of NO may contribute to impaired cytotrophoblast invasion that is seen in pre-eclampsia.

In recent years, NO has become a promising therapeutic target for cardiovascular diseases such as atherosclerosis that are associated with oxidative stress (30). Many studies have shown that endothelium-derived NO acquires an atheroprotective effect. Research has shown that responses to nitrogylcerine cause vasodilation in coronary arteries which indicates the smooth muscle is responsive to NO. Furthermore, studies have examined the possibility of reversing endothelial dysfunction by enhancing the release of NO from the endothelium by stimulation of NOS or by protecting NO from oxidative inactivation (31).

Sildenafil citrate is a cGMP specific phosphodiesterase inhibitor, which potentiates the action of NO by inhibiting the degradation of cGMP. Herraiz et al examined perinatal outcome after administration of sildenafil in a pre-eclampsia model in rats (32). Sildenafil was shown to restore normal values of blood pressure and reduce proteinuria in a rat model of pre-eclampsia. Similarly, sildenafil has been shown to reduce plasma levels of sFlt-1 and sEng, in pre-eclamptic (l-NAME induced) Sprague–Dawley rats (33). These two studies show the potential of sildenafil as a protective therapy for pre-eclampsia. However, in a small placebo controlled RCT of sildenafil in women with established pre-eclampsia showed no significant difference in time from randomisation to delivery in the two groups. (34). However, there was a small increase in birth weight in the babies born to women in the sildenafil arm. Sildenafil may work by increasing uteroplacental circulation and perfusion which improves gaseous and nutrient exchange, improving fetal growth.

In vitro, sildenafil modifies the function of myometrial arteries but not the omental and chorionic arteries in placental bed biopsies from women with pre-eclampsia (34). Sildenafil may not work in the treatment of pre-eclampsia due to the omental and chorionic arteries not being NO responsive. However, the effect that was seen was greater in IUGR than PE, suggesting chorionic plate vessels might be more relevant to the pathophysiology of PE. There is evidence from *ex vivo* and animal models of growth restriction that sildenafil citrate increases average pup birth weight and improves uteroplacental blood flow (35). As a result this drug is now being investigated in a randomised double blind, placebo-controlled clinical trial (the STRIDER study) to quantify the effects of administration of sildenafil on pregnancy outcome in severe early-onset FGR (36).

Selenium

Glutathione peroxidase (GPx) and thioredoxinreductase (ThxRed) are selenoproteins with a selenium atom (Se) integrated in their active site in the form of selenocysteine (37). This integration is essential for complete catalytic activity and as a result the activity and expression of these proteins are extremely sensitive to the amount of selenium present (38). *In vivo* studies have shown that rats develop a pre-eclampsia-like syndrome following withdrawal of selenium in their diet (39). Serum concentrations of selenium, expression and activity levels of glutathione peroxidase in maternal and umbilical venous blood samples were significantly reduced (40). However, selenium concentrations were further reduced in umbilical venous samples in pre-eclampsia patients, (40). The functional role of selenoproteins was examined in relation to protecting trophoblast cells from oxidative stress by up-regulating the selenoproteins GPx and ThxRed (37). Cells were treated with either H_2O_2, t-butyl $H_2O_2$and cumene-H_2O_2 respectively to induce oxidative stress. The study showed all 3 forms of peroxide induced cellular stress and this increase could be reversed by supplementation with both inorganic and organic forms of selenium. Watson et al, concluded that Se mediates its protective effects through up regulation of the antioxidant enzyme activity of GPx and ThxRed which in turn increases the trophoblast capacity to tolerate oxidative stress (37). More recently, the effects of selenium on markers of risk of pre-eclampsia in pregnant women was examined in a pilot randomised, control trial (41). 230 women were randomised and given either 60 µg/d (re-enriched yeast) or placebo from 12-14 weeks' gestation until delivery. Whole blood selenium concentrations were measured at both baseline and 35 weeks gestation and furthermore, plasma selenoproteins P (SEPP1) concentrations were examined at 35 weeks' gestation. This study showed that between 12 and 35 weeks gestation, whole blood selenium concentrations were increased significantly in the selenium

treated women opposed to the placebo group. Furthermore, SEEP1 concentrations measured at 35 weeks were also significantly higher in the treatment group than the placebo group.

Lifestyle

Lifestyle modifications including exercise, weight loss, diet change and reduced salt intake improve endothelial function, protect against vascular disease, lower blood pressure and might also lead to reduced vascular complications in patients with hypertension (42). Prolonged exercise activates the Nrf2 pathway leading to the up-regulation of endogenous antioxidants including SOD, glutathione and peroxiredoxin (43). However, the evidence to support advice on exercise for pregnant women with hypertensive disorders in pregnancy is limited. According to the American College of Obstetrics and Gynaecology (ACOG), pre-eclampsia is an absolute contraindication to aerobic exercise in pregnancy (44) as opposed to RCOG which advises caution while exercising with medical disorders in pregnancy (45). Studies which have investigated whether exercise in pregnancy reduces the risk of pre-eclampsia, analysis suggests a reduced risk of preeclampsia with increasing levels of physical activity before pregnancy and during early pregnancy (46). A recent study has shown a trend towards a protective effect of exercise and the occurrence of pre-eclampsia (47).

Mitochondria-targeted antioxidants

Mitochondria are the central cellular source of ROS. The placenta is a highly metabolic organ with vast numbers of functional mitochondria to manage the increasing demands of pregnancy. Mitochondrial dysfunction is a pathogenic mediator of oxidative stress and there is circumstantial evidence linking mitochondrial dysfunction with pre-eclampsia (48). Research has shown elevated mitochondrial lipid peroxidation and increased evidence of susceptibility to oxidation in mitochondria of pre-eclamptic placentas (49). Furthermore, increased activity of the placental mitochondrial electron transport chain in preterm pre-eclamptic patients compared to normotensive controls has been reported (50). Alpha-1-mjcroglobulin (A1M) is a scavenger of free heme and radicals and protects cells against haemoglobin and heme induced oxidative damage. Increased levels of A1M have been reported in plasma and urine of women with pre-eclampsia (51). Furthermore, A1M binds to the mitochondrial complex 1 subunit NDUFAB1 and protects against mitochondrial swelling upon exposure to heme and ROS (52).

Recent work from our group demonstrated increased production of mitochondrial-specific superoxide in human umbilical vein endothelial cells (HUVEC) treated with pre-eclampsia plasma samples compared with normotensive controls (53). The field of mitochondrial pharmacology has greatly progressed recently with a number of different pharmacology approaches in development, to tackle mitochondrial dysfunction. Interestingly, there is substantial evidence demonstrating that mitochondrial-targeted antioxidants may alleviate the clinical characteristics of pre-eclampsia. Recent work identified that mitochondria-targeted antioxidants (Mito-Tempo) alleviated endothelial dysfunction, increased vascular nitric oxide production, reduced mitochondrial superoxide and subsequent hypertension in two *in vivo* models of hypertension (54). Mito-tempo reduced mROS and increased vasodilation of visceral adipose arteries from morbidly obese subjects highlighting its potential to successfully treat pre-eclampsia in this obese cohort (55).

Based on the evidence discussed above (and summarised in Figure 1), oxidative damage is a significant pathological mediator of pre-eclampsia and therefore it is critical to identify therapeutics targeting this process.

Conclusions

Oxidative stress occurs when the generation of ROS overpowers the fundamental antioxidant defences. In normal pregnancy the production of ROS increases towards the end of gestation, however antioxidant volume increases in order to maintain oxidative balance. In this review, we provided evidence of elevated oxidative stress and its role in the pathogenesis of pre-eclampsia. We also discuss novel antioxidant targets that warrant further investigation before entering the clinical arena as therapies. Future directions for potential treatments of pre-eclampsia should possibly target oxidative stress through mitochondrial-targeted antioxidants and selenium supplementation.

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