Can the biology of VEGF and haem oxygenases help solve pre-eclampsia?

Asif Ahmed*†[1] and Melissa J. Cudmore*†

*Department of Reproductive and Vascular Biology, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, U.K. and †Birmingham Women's Hospital, Edgbaston, Birmingham B15 2TG, U.K.

Abstract

Pre-eclampsia, a pregnancy-specific multi-organ syndrome characterized by widespread endothelial damage, is a new risk factor for cardiovascular disease. No therapies exist to prevent or treat this condition, even to achieve a modest improvement in pregnancy length or birth weight. Co-administration of soluble VEGFR-1 [VEGF (vascular endothelial growth factor) receptor-1; more commonly known as sFlt-1 (soluble Fms-like tyrosine kinase-1)] and sEng (soluble endoglin) to pregnant rats elicits severe pre-eclampsia-like symptoms. These two anti-angiogenic factors are increased dramatically prior to the clinical onset of pre-eclampsia and are quite possibly the 'final common pathway' responsible for the accompanying signs of hypertension and proteinuria as they can be reversed by VEGF administration in animal models. HO-1 (haem oxygenase-1), an anti-inflammatory enzyme, and its metabolite, CO (carbon monoxide), exert protective effects in several organs against oxidative stimuli. In a landmark publication, we showed that the HO-1 pathway inhibits sFlt-1 and sEng in cultured cells and human placental tissue explants. Both CO and NO (nitric oxide) promote vascular homoeostasis and vasodilatation, and activation of VEGFR-1 or VEGFR-2 induced eNOS (endothelial nitric oxide synthase) phosphorylation, NO release and HO-1 expression. Our studies established the HO-1/CO pathway as a negative regulator of cytokine-induced sFlt-1 and sEng release and eNOS as a positive regulator of VEGF-mediated vascular morphogenesis. These findings provide compelling evidence for a protective role of HO-1 in pregnancy and identify it as a target for the treatment of pre-eclampsia. Any agent that is known to up-regulate HO-1, such as statins, may have potential as a therapy. Any intervention achieving even a modest prolongation of pregnancy or amelioration of the condition could have a significant beneficial health impact worldwide.

Introduction

Pre-eclampsia is a pregnancy-specific multi-organ syndrome characterized by widespread endothelial damage with a clinical presentation of hypertension and proteinuria after 20 weeks gestation [1]. It affects 3–8% of all pregnancies, with an incidence of 0.8% before 32 weeks [2]. If left unmanaged, pre-eclampsia can develop into eclampsia, an acute and life-threatening complication characterized by the appearance of tonic–clonic seizures. Even though eclampsia was first described 4000 years ago, pre-eclampsia and eclampsia still complicate up to 10% of pregnancies and both their cause and cure remain elusive. The WHO (World Health Organization) reports that over 60000 maternal deaths occur worldwide annually as a consequence of pre-eclampsia and ~12% of affected babies die within the first month. Pre-eclampsia is responsible for 30% of all premature deliveries and is associated with approx. 4 million IUGR (intrauterine growth restriction) babies. Moreover, pre-eclampsia also has a long-term impact on health. Systematic review and meta-analysis showed that women with pre-eclampsia are at a 2-fold increased risk of developing cardiovascular disease later in life [3]. Thus the global social and economic burden of pre-eclampsia is immense and still the only effective therapy is delivery of the baby and placenta.

Key words: carbon monoxide, haem oxygenase-1 (HO-1), pre-eclampsia, soluble endoglin (sEng), soluble Fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF).

Abbreviations used: eNOS, endothelial nitric oxide synthase; HO-1, haem oxygenase-1; IUGR, intrauterine growth restriction; PlGF, placental growth factor; sEng, soluble endoglin; sFlt-1, soluble Fms-like tyrosine kinase-1; SOD, superoxide dismutase; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; VEGFR-1, VEGF receptor-1.

[1]To whom correspondence should be addressed (email a.s.ahmed@bham.ac.uk).

Pathophysiology of pre-eclampsia

Increasing evidence supports our original premise that loss of VEGF (vascular endothelial growth factor) activity, potentially by elevation of sFlt-1 (soluble Fms-like tyrosine kinase-1), might contribute to the maternal symptoms of pre-eclampsia [4]. Recent studies have proven that serum levels of sFlt-1, PlGF (placental growth factor) and sEng (soluble endoglin) give the highest strength of association with outcome [5,6]. Studies in rats provide direct evidence that excess sFlt-1 plays a role in the pathogenesis of pre-eclampsia. Adenoviral delivery of sFlt-1 to pregnant rats mimics the clinical manifestations of pre-eclampsia [7]. Interestingly, the administration of sFlt-1 or a VEGF neutralizing antibody to non-pregnant rats also results in glomerular endothelial cell damage and proteinuria [8]. We showed that hypoxia and VEGF stimulate the release of sFlt-1 from endothelial cells and placental explants and that elevated sFlt-1 generated by pre-eclamptic placenta is responsible for suppressing

angiogenesis [9]. More importantly, we demonstrated that elevated sFlt-1 from pre-eclamptic placenta was unlikely to be due to hypoxia itself *in utero*, as the pre-eclamptic placenta continued to generate substantially higher levels of sFlt-1 into the conditioned media even 24 h *ex vivo* when cultured under atmospheric conditions, as compared with normal pregnancy placental explants [9]. This finding challenges the more traditional belief that pre-eclampsia arises due to placental hypoxia since it would appear that the clinical signs of pre-eclampsia are due, in large part, to elevated levels of sFlt-1. In collaboration with Weich's laboratory, we subsequently showed that introduction of sFlt-1 into pregnant mice, which was accompanied by hypertension and proteinuria, could be reversed by VEGF [10]. This demonstrates that below a critical threshold sFlt-1 fails to elicit damage to the fenestrated endothelium of the kidney [10] and could explain why fetal growth-restricted pregnancies, with sFlt-1 levels elevated above those in normal pregnancies, are not accompanied by hypertension or proteinurea. We also know that cancer patients receiving anti-VEGF therapy (Avastin) exhibit pre-eclampsia-like symptoms, suggesting that decreased bioavailability of VEGF causes these symptoms [11], as may be the case in pre-eclampsia. Furthermore, uteroplacental ischaemia in primate or rodent pregnancy models produced clinical signs analogous to human pre-eclampsia, including increased circulating sFlt-1 [12,13]. Previously, a cleaved form of TGF-β (transforming growth factor-β) co-receptor, endoglin (sEng), was shown to act synergistically with sFlt-1 to induce endothelial dysfunction and the HELLP (haemolytic anaemia, elevated liver enzymes and low platelet count) syndrome (microangiopathic haemolytic anaemia and thrombocytopenia) in pregnant rats [14]. sEng is elevated in the serum of pre-eclamptic women 8–12 weeks prior to the clinical onset of the disease [6]. Signalling by TGF-β, which has both anti-inflammatory and atheroprotective properties [15] and activates eNOS (endothelial nitric oxide synthase), may be disrupted by sEng [14] (Figure 1). Any intervention that would reduce the prevalence of these circulating factors may not only prolong the pregnancy but also protect the mother from permanent vascular damage.

Sflt-1 in vascular homoeostasis

VEGF is not only critical for angiogenesis but also promotes survival of the endothelium by activating Akt (also known as protein kinase B) and eNOS [16]. Previously, we showed that VEGF utilizes alternative signalling pathways via VEGFR-1 (VEGF receptor-1) or VEGFR-2, which converge on Akt to promote eNOS activation and angiogenesis [17]. Our idea that failure to generate sufficient NO might predispose women to pre-eclampsia or fetal growth restriction [4,18] may hold true as these conditions may reflect impairment of VEGF-dependent eNOS activation in the endothelium. sFlt-1 acts as a potent inhibitor of VEGF-mediated biological activities through sequestration of VEGF [19] and formation of dominant-negative complexes with full-length VEGFRs [20] (Figure 2).

Figure 1 | Vascular dysfunction in pre-eclampsia

A functional endothelial monolayer requires VEGF and PlGF binding to their full-length receptors (VEGFR-1 and VEGFR-2), on the endothelium, and initiating signalling cascades. In pre-eclampsia, this protective signal is compromised due to an excess of sFlt-1, which is compounded by a decrease in the expression of PlGF and a rise in circulating sEng.

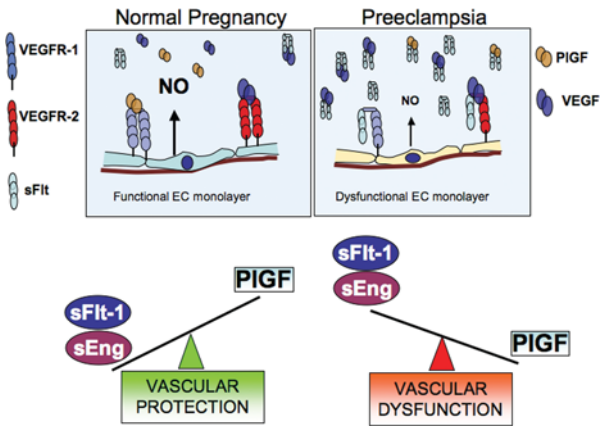
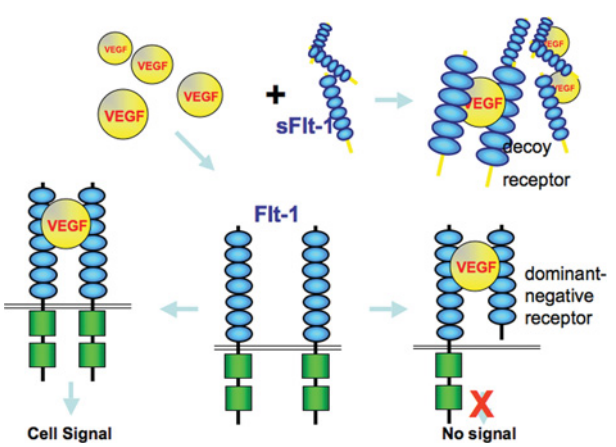


Figure 2 | sFlt-1 prevents VEGF signalling by two mechanisms

sFlt-1 binds free VEGF and PlGF and thereby prevents their binding to full-length receptors on the endothelium. sFlt-1 also forms dominant-negative complexes with membrane-bound VEGFRs; hence, although VEGF can continue to bind to the cell surface, no signal is initiated.



Predictability of pre-eclampsia

No definitive biomarker exists with the potential for risk stratification or delineation of the mechanism of action for potential therapeutic agents. The only effective treatment for pre-eclampsia resolution is the delivery of the placenta, demonstrating that the disease is of placental origin [1]. As pre-eclampsia has a long preclinical phase, a predictive test would allow efficient assessment of potential therapeutic interventions, permit a targeted approach concentrating on women at the highest risk and release valuable resources. Previous studies demonstrate that serum levels of sFlt-1, PlGF and sEng give the highest strength of association with

outcome [5,6]. The combination of these three biomarkers is likely to afford the greatest accuracy in predicting early onset pre-eclampsia, and mid-gestational screening would be appropriate to identify pregnancies at high risk in need of special follow-up to ensure timely delivery if pre-eclampsia develops.

Vascular protection in pre-eclampsia

The use of antioxidant supplements, as shown by the VIP (Vitamins In Preeclampsia) trial, is insufficient to regulate the oxidative status of the placenta and to prevent pre-eclampsia in women at risk [21], and our previous study showed that vitamin C and vitamin E supplementation had no effect on sFlt-1 or sEng levels [22]. In 2000, Ahmed et al. [23] stated that vascular protective factors regulate pregnancy and are responsible for resolution of the exacerbated inflammation associated with pre-eclampsia and that it is the loss of these endogenous factors that predisposes women during pregnancy to pre-eclampsia.

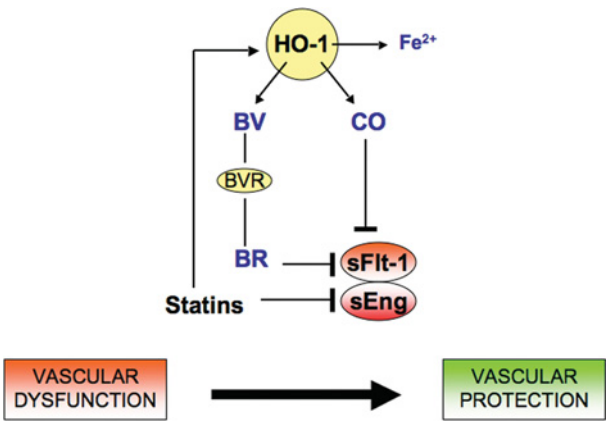
Therapies that can enhance the activity of major endogenous protective/antioxidant factors, such as HOs (haem oxygenases), the thioredoxin system, SOD (superoxide dismutase) and glutathione peroxidase, may prove effective. In particular, there is mounting evidence that the stress response gene HO-1 and/or its catalytic product, carbon monoxide, confer cytoprotection against tissue and cellular injury [24,25]. In fact, HO-1 is up-regulated after reperfusion and is widely accepted to protect against ischaemia/reperfusion injury [25]. Indeed, we showed that TNFα (tumour necrosis factor α)-mediated cellular damage in placental villous explants could be prevented by up-regulating HO activity by haemin, supporting our proposition that the resolution of oxidative stress and inflammation associated with pregnancy is controlled by placental HO and it may be the lack of such a compensatory system that leads to pre-eclampsia [23]. Recently, we went on to show that HO pathways inhibit the release of sFlt-1 and sEng in the placenta and the endothelium and thus may provide protection to the maternal endothelium to prevent pre-eclampsia [22] (Figure 3). Using an animal model of pre-eclampsia, we concluded that decreasing the circulating levels of free sFlt-1 below a critical threshold minimizes hypertension and proteinurea associated with overexpression of sFlt-1 [10]. We hypothesize that reducing the circulating levels of free sFlt-1 below this threshold value in women with pre-eclampsia will alleviate the clinical signs of the condition, and that this could be achieved by the use of statins or CO therapy (Figure 3).

Potential new therapies

At present, the only effective treatment for pre-eclampsia is delivery. However, early delivery, especially before 32 weeks gestation, can have serious consequences for the health of the baby, whereas 'watchful waiting', often employed to allow for fetal lung maturity *in utero*, increases maternal risks. There is no effective treatment to prevent pre-eclampsia, and current management therapies have limitations. Low-dose aspirin

Figure 3 | HO-1/CO could tip the balance to a vascular protective phenotype in pre-eclampsia

HO-1 degrades haem to produce biliverdin, CO and free iron. Biliverdin is rapidly converted into bilirubin by biliverdin reductase (BVR). Genetic studies have established that HO-1 and BVR can prevent the overexpression of sFlt-1 and sEng from the endothelium. Furthermore, the cholesterol-lowering drugs, statins, induce HO-1, which, if sufficient substrate is available, would lead to a lowering of circulating anti-angiogenic factors, thus rescuing dysfunctional or damaged endothelium.



is the only prophylactic therapy for primary prevention, but only mildly reduces the risk of future pre-eclampsia in high-risk women [26]. A cheap, effective and safe therapy for prevention of pre-eclampsia complications is urgently needed. Agents capable of substituting for the deficiency or inducing the activity of the HO system and/or reducing the elevated sFlt-1/sEng may have therapeutic potential for alleviating the severity of pre-eclampsia and in turn prolonging pregnancy in early onset disease, thus reducing the complication burden for both the mother and newborn.

CO

Despite the well-known adverse effects of smoking during pregnancy (spontaneous abortion, stillbirth, preterm labour, IUGR, placenta previa and placental abruption), smokers, paradoxically, have a 32% decreased incidence of pre-eclampsia [27]. However, snuff (smokeless tobacco) users have an increased incidence [28], indicating that it is a combustible product of tobacco that confers the protection. Indeed, smokers have reduced circulating sFlt-1 [6]. Cigarette smoke extract induces HO-1 expression in trophoblasts [29] and decreases sFlt-1 release from placental villous explants without altering placental apoptosis status [30]. Furthermore, CO treatment enhances SOD and HO-1 expressions [31].

There is mounting evidence that the stress response gene HO-1 and/or its catalytic by-product CO confer cytoprotection against tissue and cellular injury [24,25]. Indeed, the enzyme generates three molecules (biliverdin, Fe^{2+} and CO), which are unique in that they all have biological activity. Biliverdin is an antioxidant, which is rapidly reduced by biliverdin reductase to bilirubin, another

potent antioxidant. We hypothesized that HO-1-/HO-2-mediated CO release may protect against pre-eclampsia via its anti-inflammatory and anti-apoptotic effects [23]. Oxidative stress-induced placental injury and subsequent release of placental debris into the maternal circulation are reported to contribute to pathogenic events in the progression of pre-eclampsia [1]. Importantly, CO inhibits hypoxia/re-oxygenation-induced apoptosis and secondary necrosis in syncytiotrophoblasts [32]. Women with pre-eclampsia have significantly decreased CO concentrations in their exhaled breath compared with those with healthy pregnancies, indicating a decreased HO activity [33,34]. Consistent with these findings HO-1 [23] and HO-2 [35–37] are down-regulated in pre-eclamptic placenta and HO-1 mRNA is decreased in the blood of pre-eclamptic women at term [37a]. Strikingly, chorionic villous samples (fetal placental cells) from women at just 11 weeks of gestation who went on to develop pre-eclampsia showed decreased HO-1 mRNA [37b], opening up the possibility that this very early decrease in HO-1 could lead, in part, to the elevated anti-angiogenic factors seen subsequently in pre-eclamptic pregnancies and provide a novel early biomarker. The landmark paper published in 2007 showed that CO or overexpression of HO-1 inhibits the production of sFlt-1 and sEng [22] supports this possibility.

Statins

Statins, a class of lipid-lowering drugs, substantially decrease cardiovascular morbidity and mortality [38] and have beneficial properties in a variety of pathological settings. Currently, statins are contraindicated in pregnancy. However, a recent observational study on the use of statins in the first trimester of pregnancy, which looked at 288 women, found no adverse effects [39]. Recently, we showed that statins inhibit cytokine-mediated release of sFlt-1 [22]. Administration of statins to mice was shown to induce HO activity, increase the formation of CO and bilirubin and increase the plasma antioxidant level [40]. Importantly, increased antioxidant levels were abolished by treatment with an HO inhibitor, suggesting that HO may mediate the beneficial pleiotropic actions of statins. These findings imply that the induction of HO and the production of CO and/or bilirubin may be a mechanism by which statins exert antioxidant actions and confer cardioprotection *in vivo*.

Pleiotropic mechanisms beyond the reduction of plasma cholesterol have been repeatedly shown to contribute to the anti-atherogenic and tissue-protective properties of statins. Statin actions, independent of cholesterol lowering, have been shown to reduce the incidence of diabetes [41] and also to reduce heart [42] and kidney transplant rejection [43], to increase survival rate of transplant recipients and to ameliorate rheumatoid arthritis [44] and multiple sclerosis [45]. These conditions all have a substantial exacerbated inflammatory component, which parallels pre-eclampsia, and these findings indicate that statins have anti-inflammatory properties. In addition, statins have a positive effect on many endogenous vascular protective proteins. They cause an increase in activity of the thioredoxin system [46], of SOD [47] and of glutathione peroxidase [48]. Statins also improve factors that are compromised in pre-eclampsia such as NO bioavailability, VEGF and endothelial progenitor cells [22,49]. It may be that statins and CO would need to be given in combination for best outcome; this is something that needs to be evaluated.

Moving forward

Our proposed mechanism implies that supplementation with statins or periodic inhalation of CO could prevent or reduce development of the signs of pre-eclampsia. Our hypothesis needs to be tested in animal models and in clinical trials and these are under way. Although pre-eclampsia is a human-specific condition, animal model use will test whether the therapies planned are effective in reducing signs of pre-eclampsia without adversely affecting fetal outcome. Numerous groups have shown that either sFlt-1 overexpression or arterial ligation to reduce uterine placental perfusion (the RUPP model) closely mimics the clinical signs of pre-eclampsia. There can be no dispute that the rodent placental biology is very different from human pregnancy and that the mouse is unlikely to be a good model to study 'human placental function'. It can, however, provide models that mimic the maternal signs of pre-eclampsia, providing a reasonable method to test the proof of concept for the hypotheses outlined herein.

A proof of principle randomized placebo-controlled trial is needed in women who develop severe early onset pre-eclampsia. In order to obtain the number of cases necessary, a multicentre trial is needed where participants are randomly allocated to receive a statin or matched placebo, CO or air or a combination of a statin and CO. We have initiated this for statin use and are currently awaiting MHRA (Medicines and Healthcare Products Regulatory Agency) approval for our MRC (Medical Research Council)-funded StAmP trial (Statins to Ameliorate early onset Pre-eclampsia). This study will assess the feasibility of a definitive trial by examining recruitment rates, compliance and physician and patient readiness for randomization to treatments, as well as changes in the pro- and anti-angiogenic profile. This initial study will identify a novel intervention based on a strong scientific rationale, which will inform a future large-scale multicentre randomized trial. Once an accurate predictive test is designed and established, women destined to develop early onset pre-eclampsia could be offered preventative statin or CO therapy or both depending on the outcome of the proof of principle and animal studies. If these studies prove successful, then the therapy could be offered to women destined to develop milder forms of the disorder.

This comprehensive approach will allow us to test whether modulation of endogenous protective factors can reduce the severity of pre-eclampsia and determine the mechanisms involved, which will lead to refinement and development of further therapeutic targets.

Conclusion

Our hypothesis proposes that activation of endogenous protective factors via CO or statins could lead to prolongation of pregnancy, which would seriously improve the outcome for mothers and babies worldwide and prevent or reduce the lifelong negative health impacts of having pre-eclampsia. We envisage a new era of pre-eclampsia management, where women at risk of developing pre-eclampsia and the severity of their condition can be accurately identified with a predictive test via a point-of-care device. Based on this information, these women can be treated with statins and/or CO therapy to alleviate the signs, prolong their pregnancies and reduce the long-term morbidity burden associated with this condition. If we are successful, statins in particular, which are cheaply available around the world, could be used in the developing world where there is major maternal and infant mortality associated with pre-eclampsia. Any intervention achieving a modest improvement in mean pregnancy length or birth weight will improve the lives of tens of thousands of women of child-bearing age and their offspring.

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