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Treatment of Pre-eclampsia: Implementing Research Findings

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Abstract

Pre-eclampsia is a complex disease that affects both mother and their developing fetus. Due to the multifactorial nature of the disease and its undeciphered etiology, we explore the possibility of improving intrinsic vasodilatory mechanisms as a method of treatment. Reduced utero-placental blood flow seems to be central in the manifestation of this disease leading to the secretions of various factors that maintain and/or worsen the condition. We and various other researchers have showed that improved placental perfusion shows promise in alleviating many symptoms of the disease. This serves as an excellent option at reducing both perinatal and maternal morbidity and mortality irrespective of the predisposing factors for the disease.

Keywords: Pre-eclampsia; Placental perfusion; Selective vasodilation

Introduction

Pre-eclampsia, is one of the most widely investigated conditions relating to human reproduction. To date no firm cure has been found, and a clear, well-defined mechanism has not been ascribed to the pathogenesis of the disorder. Some researchers seem to focus on single pathways in isolation of others. The disease rather represents a multitude of possible underlying pathologies involving genetics, immune dysregulation, vascular maladaptation and sociobiological factors; complicating clinical management. However, a central theme is the presence of reduced placental perfusion resulting in a hypoxic and/or ischaemic placenta, delivery of which results in a resolution of clinical symptoms. It is within this context that we examine how an intervention such as increasing placental perfusion may represent a promising treatment strategy for reducing maternal and neonatal mortality and morbidity related to the pre–eclampsia/ eclampsia syndrome in low resource environments.

Definition, Incidence and Classification of Pre-eclampsia

Definition

Pre-eclampsia is a specific disorder of human pregnancy, which is commonly characterised by hypertension and proteinuria after 20 weeks of gestation. It should however be recognised as a multi system disorder in which one organ system may also be predominantly affected. Thus, the disorder may present as isolated thrombocytopenia or intrauterine growth restriction (IUGR). [1-3]. Steegers et al. [4] also makes the point that pre-eclampsia, sometimes progresses into a multi-organ cluster of varying clinical features.

Incidence

The worldwide incidence of pre-eclampsia is estimated to be approximately 8,370,000 cases per annum [3]. Each year, 814,000 neonatal deaths and 1.02 million stillbirths result from intrapartum-related causes, such as intrauterine hypoxia with pre-eclampsia being one of the major risk factors. Thus the disease is said to be the leading cause of both maternal and fetal morbidity and mortality, especially in low and middle income countries (LMIC) [3,5-7]. Locally, 84% of the 622 deaths linked to hypertensive disorders in pregnancy between 2005 and 2007 were due to the pre-eclampsia/ eclampsia syndrome [8]. Furthermore, the incidence of hypertension in pregnancy, in a population based study in this Province was 12.5% in 2004. In sub-Saharan Africa, recent health services assessments found that only 15% of all hospitals where equipped to provide basic neonatal resuscitation, [9] and with increased daily admissions of pre-eclamptic patients this is an added burden to healthcare in LMIC countries. Maternal deaths from hypertensive disorders in pregnancy are also a common in high income countries [7], and is the foremost cause for the admission of pregnant women into intensive care units [2]. Clearly a simple but effective intervention is urgently needed.

Classification

Because of the multifactorial pathogenesis of the different pre-eclampsia phenotypes, classification has been somewhat difficult [10]. Despite this, Wagner [11] has attempted to classify the different hypertensive disorders of pregnancy into four main types; chronic hypertension gestational hypertension, pre-eclampsia superimposed on chronic hypertension and pre-eclampsia (Figure 1). Basically, chronic hypertension refers to the presence of hypertension before 20 weeks of gestation in the absence of, or stable proteinuria. Gestational hypertension refers to hypertension after 20 weeks of gestation without proteinuria. A patient with hypertension before 20 weeks gestation, and a further increase in blood pressure; along with de-novo proteinuria or a sudden increase in existing proteinuria, may be diagnosed as having pre-eclampsia superimposed on chronic hypertension [11].

Etiology and Pathogenesis of Pre-eclampsia

The etiology and pathophysiology of pre-eclampsia is still not clearly understood, however many accept a two-stage model of the disease. It is known that the pathogenic process begins much earlier than the symptoms; perhaps at the onset of trophoblastic invasion and remodelling of the spiral arteries during the first trimester of pregnancy [12]. Therefore, the first stage is vascular maladaptation

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in the placental bed, due to the failure of the uterine spiral arteries to undergo complete remodelling into wide bore channels; an important vascular modification in normal pregnancies [1,13-15]. This maladaptation is associated with a marked reduction in blood flow to the placenta. The second stage, the maternal stage, is one in which the reduced placental perfusion results in release of a variety of substances; including trophoblastic debris; necrotic tissue; and an excess secretion of factors, which affect virtually every major organ system through endothelial dysfunction and systemic vasospasm. These events are depicted sequentially in Figure 2.

Reduced Uterine and Placental Perfusion

A key event in the etiopathology of the disease is considered to be the inability of the extravillous trophoblast (EVT) to change their phenotype to a more invasive type which is needed for vascular remodelling. The arteries fail to remodel into wide bore "compliance" vessels, offering little resistance but they rather act as resistance vessels resulting in a marked reduction in blood flow to the placenta [1,2,14]. What exactly causes these EVT to display this aberrant behaviour is not clear. The resulting hypoxic placenta secretes excess soluble fms-like tyrosine kinase-1 (sFlt1) into maternal circulation [16]. This excess sFlt1 has been shown contribute to hypertension, proteinuria, endothelial dysfunction and IUGR, which are classic phenotypes of this disease [17]. The increased circulating level of sFlt-1 is thought to play a role in the development of hypertension by opposing the physiological effects of NO-dependant vasodilation [18]. Others have suggested that elevated circulating sFlt-1 may also result in proteinuria by down regulating renal nephrin [19]. Since placental and fetal weights are strongly correlated with uteroplacental blood flow it certainly accounts for the IUGR [20].

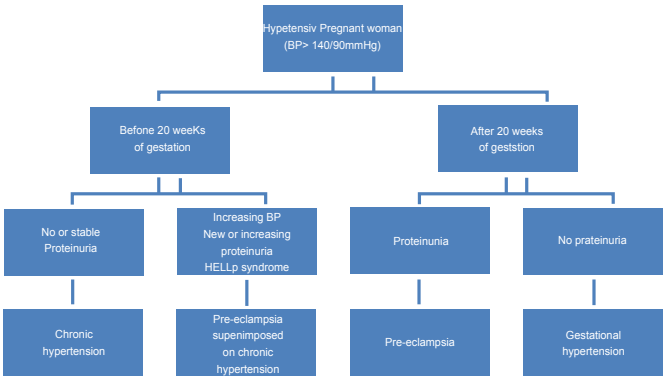

Figure 1: Differentiation of the hypertensive disorders of pregnancy [11].

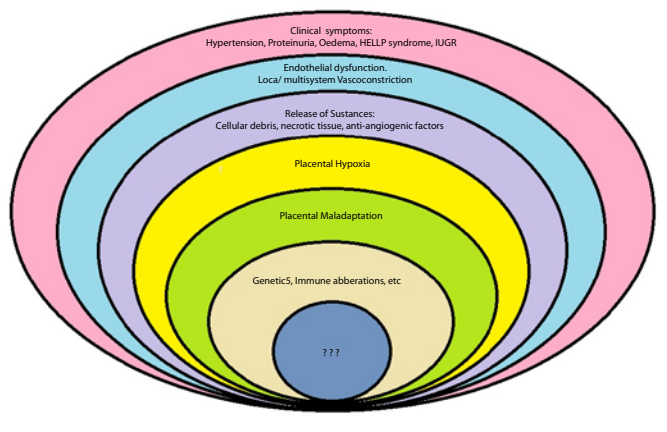

Figure 2: The onion model for the etiology of pre-eclampsia.

Substances that can Alter Uterine Vascular Dilation

Valdes et al. [21] proposed that there are five vasodilatory factors/systems that have a functional role in maintaining normotension during pregnancy. These five are prostacyclin, nitric oxide, kallikrein, angiotensin-(1–7) and VEGF-A. The expression of these vasodilators either in the different trophoblastic subtypes, or in the fetal endothelium in humans, rats, guinea-pigs and sheep, suggests that they oppose certain vasoconstrictor systems *in vivo,* or participate directly in vascular remodeling.

Prostanoids

The role of prostanoids in pregnancy was investigated as early as the 1970's, with the two main protagonists of the system being prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) [22-24]. Both vasoactive factors are synthesized from prostaglandin (PGH_2), which is ultimately metabolised from arachidonic acid under the influence of the enzymes, constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2). Prostacyclin synthase and thromboxane synthase are the two enzymes responsible for the synthesis of PGI_2 and TXA_2 respectively. The distribution of these enzymes therefore makes this cascade very cell specific.

Prostacyclin is a major vasodilator, synthesized from the endothelium, [25] whereas TXA_2 is a potent vasoconstrictor and procoagulant derived from platelets. PGI_2 thus mediates its effects directly on the smooth muscle of blood vessels or by opposing the effects of TXA_2. In normal pregnancies the excretion of urinary metabolites of PGI_2 demonstrates a steady and marked rise with almost a fivefold increase near term, however the urinary excretion of thromboxane metabolites remains unchanged, indicating a net vasodilatory effect. In preeclampsia, decreased urinary excretion of the PGI_2 metabolites are observed from as early as 13 weeks of pregnancy [26] and elevated TXA_2 levels are seen after 21 weeks, ultimately favouring the vasoconstrictor effect. This decreased PGI_2 levels and increased TXA_2 levels have been attributed to increased lipid peroxidation and decreased scavengers in preeclamptic patients [27]. These findings initiated a large scale clinical trial using low dose aspirin; a cyclooxygenase inhibitor, which only showed a modest reduction in the incidence of the disease [28].

Nitric oxide

Nitric oxide is produced by the deamination of L-arginine into citrulline and NO in the presence of the enzyme nitric oxide synthase (NOS) [29]. There are 3 isoforms of this enzyme, i.e. endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). In the case of vasculature, eNOS is the dominant isoform [29,30]. Nitric oxide exerts its vasodilatory effects through the action of its second messenger, cyclic guanidine monophosphate (cGMP), which is produced as a result of NO interaction with an iron molecule in the enzyme guanylyl cyclase to ultimately phosphorylate guanidine triphosphate (GTP) into cGMP [29].

The role of NO in pregnancy has been widely reported, however in this review we allude to a few pertinent studies. Plasma and urinary levels of nitrate, and urinary levels of cGMP are shown to be increased throughout pregnancy [31,32].Exogenous blockade of NO synthesis in animal models has been shown to mimic the effects of pre-eclampsia [33,34]. In addition, elevated levels of the endogenous NOS inhibitor, dimethylarginine (ADMA), in the second trimester of pregnancy, is

associated with endothelial dysfunction, impaired uterine artery blood flow and the subsequent development of preeclampsia [35].

Further evidence for the importance of NO in pregnancy was demonstrated in a few clinical trials where hypertensive pregnant patients were supplemented with L-arginine (NO substrate) and exhibited an improvement in both systemic and placental perfusion, coupled with an improvement in blood pressure [36-39]. These findings certainly warrant further investigations with L-arginine supplementation. It is within this context that we chose to study sildenafil citrate as a NO donor, which yielded beneficial outcomes to pregnancies complicated by a reduction in uterine perfusion pressure (RUPP) [40].

Kallikrein-kinin system

The kallikrein-kinin system is best described as an endogenous cascade whereby the two kinins, kallidin and bradykinin, are cleaved from either low or high molecular weight kininogens by two serine proteases, namely tissue and plasma kallikrein [21]. Both kinins elicit their effect by binding to one of two receptors, kinin type 1 receptor (B1R) or kinin type 2 receptor (B2R). Kinin type 1 receptor activation promotes angiogenesis, mitogenesis and induces pain, whereas B2R activation is associated with increased vascular permeability, decreased platelet aggregation and vasodilation. The latter effects are either directly mediated by B2R activation or indirectly by the synthesis of NO and/or PGI_2 [41]. Increased urinary kallikrein excretion is associated with normal pregnancies and reaches its maximum by 8-12 weeks of gestation [42]. This rise in urinary kallikrein excretion is not observed in hypertensive pregnancies [43,44] and even lower levels are observed in patients destined for preeclampsia [45] suggesting that the kallikrein-kinin system plays a functional role in maintaining placental perfusion.

Vasodilator components of the renin-angiotensin system

The renin-angiotensin system (RAS) is an established vasoconstrictor system; however recent reports also allude to a vasodilator arm of this cascade. The latter effects are mediated through angiotensin (1-7) [ang 1-7] which act on the Mas receptor to cause vasodilation, angiogenesis, collagen production, thrombosis and inhibition of vascular smooth muscle growth. Ang 1-7 is linked to the RAS by angiotensin converting enzyme 2 (ACE-2), and may be produced by any one of three biochemical pathways. The first mechanism of ang 1-7 production is by the cleavage of one amino acid from angiotensin II [ang II] by ACE-2, propyl endopeptidase (PEP) or carboxypeptidase (CPB). Secondly, ang 1-7 may be produced by the cleavage of 3 amino acids from angiotensin I [ang I] by neutral endopeptidase (NEP) and thirdly, ang 1-7 may be produced by a two-step reaction, where ang I is converted to angiotensin 1-9 [ang 1-9] by ACE-2, followed by the cleavage of 2 amino acids by angiotensin converting enzyme (ACE) and NEP.

Urinary and plasma levels of ang 1-7 have been shown to increase throughout normal pregnancies and are reduced in pre-eclampsia, suggesting that this vasodilator plays an important role in the vascular adaptations to pregnancy [46-48]. Further evidence supporting this notion was seen in a RUPP model that mimics preeclampsia, where it was shown that ang 1-7 and ACE-2 mRNA expression was decreased in the placenta [49-51]. This suggests that RUPP associated with pre-eclampsia inhibits the vasodilator arm of RAS possibly through factors such as sFlt-1, TNF-α or angiotensin type 1 receptor auto-antibody that are secreted as a consequence of placental hypoxia and decreased placental blood flow [49-51]. The effects of ang II, mediated by the AT_1 receptor, is well documented with the most common being vasoconstriction, cell proliferation, fibrosis and angiogenesis. In contrast is the binding of ang II to the AT_2 receptor resulting in opposite effects i.e. vasodilation, antiproliferative, antifibrotic and antiangiogenic, mediated by eNOS and kinins [52-54]. The role of ang II as a vasodilator in pregnancy was highlighted in a study that showed that ang II was absent in the uterine arteries of non-pregnant sheep as compared to pregnant ewes that underwent normal pregnancy [55,56].

VEGF-A as a vasodilator

Vascular endothelial growth factor -type A (VEGF-A) is one of the 4 isoforms of vascular endothelial growth factor (VEGF) and is known to promote angiogenesis by inducing vascular permeability, cell migration and protease production by endothelial cells [57,58]. This role in vascular remodeling during placentation is shared by placental growth factor (PlGF) and angiopoietins 1 and 2 [59].

VEGF can also cause vasodilation by binding to tyrosine kinase-1 type fms receptors (VEGFR-1 [Flt-1]), which are modulated by VEGFR-2 (Flk-1/kinase domain [KDR]) receptors [58,60]. This receptor binding activates eNOS to produce NO [61] as well as PGI_2 synthesis from PGH_2 [62]. In a study conducted by Brownbill et al. [63], where VEGF was perfused in human placentas, a strong vasodilatory effect was observed in the placental vasculature, which was later shown to be mediated by NO. In another study conducted by Brockelsby et al. [64], VEGF was shown to increase PGI_2 synthesis in bovine endothelial cells thereby causing vasodilation.

As mentioned earlier, the hypoxic placenta which is characteristic of pre-eclampsia has been shown to secrete excessive amounts of sFlt1 into maternal circulation [16]. This is a soluble form of the Flt-1 receptor that is generated by alternative splicing [65]. Free VEGF therefore binds to the vast amounts of circulating sFlt1 thereby reducing its role in either vascular remodeling and/or fetoplacental vasodilation which are both crucial in normal pregnancy [21].

Current Management Strategies and Proposed Interventions

To date there is no known treatment for preeclampsia. The only known cure is the delivery of both the fetus and the placenta. However, early delivery may place the fetus at risk of prematurity and subsequent perinatal morbidity and mortality. Thus prior to fetal viability treatment is aimed at lowering high blood pressure, thus reducing maternal complications while awaiting fetal maturity. Commonly used anti-hypertensive agents cause systemic vasodilation and can only slightly improve blood pressure control, but have no significant clinical effects on improving renal function and increasing placental blood flow. It is the decreased blood flow to the placenta that ultimately leads to IUGR and a multi system endothelial dysfunction.

Since the etiology and pathogenesis of the pre-eclampsia remains elusive and given the multi-factorial complexity of the disease, investigators should therefore focus on treatments that can increase utero-placental blood flow by manipulating any of the five proposed mechanisms for uterine vascular dilation. This in essence should cause improved placental perfusion and hence decrease placental hypoxia and subsequently decrease the secretion of the anti-angiogenic factors that aggravate the disease. This would ideally offer a treatment to pre-eclampsia irrespective of the predisposing factors for the disease.

Sildenafil citrate and the nitric oxide pathway

As mentioned earlier, nitric oxide (NO) is a locally active

vasodilator that relaxes vascular smooth muscle directly through a cGMP-mediated pathway, and indirectly by inhibiting the production of vasoconstrictors including endothelin – 1 (ET_1) [29]. In recent years, animal research has demonstrated that NO leads to relaxation of vascular smooth muscles and is a powerful modulator of uterine blood flow. Furthermore, the findings of Gokina et al. (2003) suggest that any decrease in NO production would result in an increase in uterine artery myogenic tone and decreased placental blood flow, possibly resulting in placental hypoperfusion, with subsequent development of hypertension or preeclampsia.

The mechanism of action for sildenafil citrate is based on this role of nitric oxide (NO) on vascular smooth muscle relaxation [66]. Sildenafil citrate (Viagra™) is a specific type-5 phosphodiesterase (PDE) inhibitor. It acts as a competitive binding agent for this type-5 phosphodiesterase and therefore favours cGMP to cause vasodilation of the penile artery and relaxation of the corpora cavernosa to ultimately cause an erection [66].

With the discovery of the same family of specific type-5 phosphodiesterase iso-enzymes in the uterus and uterine vasculature [67,68] sildenafil citrate certainly warranted investigations into its vasodilatory effect in the female reproductive system. Researchers have also shown sildenafil citrate to improve uterine artery blood flow and endometrial development in women undergoing *in vitro* fertilization [69], as well as having beneficial effects on fetal and vascular parameters in hypertensive pregnant rats [70]. Sildenafil citrate was also shown to enhance vasodilation and improve the endothelial function of myometrial vessels in pregnancies complicated by intra-uterine growth retardation (IUGR) [71]. In a rat pre-eclamptic model, sildenafil citrate increased cGMP content in thoracic aortic muscle rings and straightened the relaxation and contraction responses, however not to control levels [72]. Studies conducted in our laboratories have shown sildenafil citrate to improve fetal outcomes, decrease proteinuria and reduce blood pressure amplification in rats with pre-eclampsia-like manifestations. We further demonstrated that sildenafil citrate decreased sFlt1 and sEng levels and showed a slight improvement in NO levels in the same model [40].

As mentioned previously, exogenous gene transfer of sFlt-1 in pregnant rats displayed various phenotypes of pre-eclampsia including hypertension, proteinuria and glomerular endotheliosis [16,17] and the co-administration of sEng in the same animal caused haemolysis and thrombocytopenia which are also notable phenotypes of the disease. Since both of these anti-angiogenic factors were decreased by sildenafil citrate, it serves as a promising treatment for pre-eclampsia and certainly warrants human trials.

Eriosema kraussianum N. E. Br. (Fabaceae)

Traditional herbal remedies form an integral part of African culture. Given the success of sildenafil citrate on pre-eclampsia-like manifestations in rats, and the expense of Viagra™, we chose to investigate the role of a plant extractive that is commonly used by South African traditional healers for erectile dysfunction (ED) [73]. This plant is classified under the genus, Eriosema (isiZulu indigenous umbrella name of "uBangalala"). The roots of Eriosema kraussianum are used by Zulu traditional healers to treat ED as follows; hot milk infusions of the plant's roots or pounded, boiled root decoctions are taken in small doses twice a day for impotence [73-75]. Two bioactive pyrano-isoflavones [Kraussianone-1 (Kr1) and Kraussianone-2 (Kr2)] were isolated from the roots of Eriosema kraussianum N. E. Br. (Fabaceae) [76,77]. Both bioactive compounds demonstrated beneficial effects in the management of ED [76,77] and further exhibited hypoglycaemic effects and vasodilatory properties in a rat model [78]. To this end, we investigated the effect of Kr2 on pre-eclampsia-like manifestations in a rat model [79]. We demonstrated that Kr2 administration improved pup survival and showed a trend toward increasing birth and placental weights. Furthermore, Kr2 administration also reduced blood pressure amplification and decreased the plasma concentrations of the two anti-angiogenic factors, sFlt-1 and sEng. We did not see an improvement in NO levels, suggesting that the plant extractive exerts a vasodilatory effect by some other mechanism. However we speculate that Kr2, by improving uterine artery blood flow, results in the improved fetal outcomes.

Conclusion

Several studies show that improved uteroplacental blood flow will reduce the symptoms of preeclampsia, irrespective of the etiopathology of the disease. Research conducted in our laboratories reinforced this concept using two different compounds that cause specific vasodilation by different mechanisms. Since both compounds improved fetal outcomes including birth and placental weights, it is plausible that there was improved placental perfusion as a result of selective vasodilation which has helped to alleviate the symptoms associated with pre-eclampsia. The promising results seen in our animal model warrants further studies whereby sildenafil citrate or kraussianone-2 should be administered at different gestation intervals; while monitoring the circulating anti-angiogenic levels.

Treatment using sildenafil citrate, should specifically target mothers that exhibit increasing levels of sFlt1 and sEng even before 20 weeks of gestation; with continuous monitoring of blood pressure, urinary protein excretion and platelet count. Given the complex multifactorial nature of this disease and the elusive nature of its eitiology, our recommendation represents a viable option to decrease perinatal and maternal morbidity and mortality from pre-eclampsia.

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