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# Matrix Metalloproteinases as Drug Targets in Preeclampsia

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## Abstract

Preeclampsia is an important syndrome complicating pregnancy. While the pathogenesis of preeclampsia is not entirely known, poor placental perfusion leading to widespread maternal endothelial dysfunction is accepted as a major mechanism. It has been suggested that altered placental expression of matrix metalloproteinases (MMPs) may cause shallow cytotrophoblastic invasion and incomplete remodeling of the spiral arteries. MMPs are also thought to link placental ischemia to the cardiovascular alterations of preeclampsia. In fact, MMPs may promote vasoconstriction and surface receptors cleavage affecting the vasculature. Therefore, the overall goal of this review article is to provide an overview of the pathophisiology of preeclampsia, more specifically regarding the role of MMPs in the pathogenesis of preeclampsia and the potential of MMP inhibitors as therapeutic options.

### Keywords

Hypertension; hypertensive disorders; matrix metalloproteinases; preeclampsia; pregnancy; therapy

## 1. INTRODUCTION

Hypertensive disorders affect up to 10% of pregnancies worldwide [1], being one of the major causes of maternal death in developed countries (~16%) and in Latin America (~26%) [2]. The National High Blood Pressure Education Program (NHBPEP) categorizes hypertension during pregnancy as follows [3]:

Preeclampsia-eclampsia: new-onset hypertension (>140 mmHg systolic or >90 mmHg diastolic blood pressure) and proteinuria (>0.3 g in 24 h) after 20 weeks of gestation in a previously normotensive women. If seizures also occur, the disease is called eclampsia;

Chronic hypertension: hypertension present before pregnancy or first diagnosed before 20 weeks of gestation;



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**CONFLICT OF INTEREST**

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Chronic hypertension superimposed on preeclampsia: new-onset or acutely worse proteinuria, sudden increase in blood pressure, thrombocytopenia, or elevated liver enzymes after 20 weeks of gestation in women with preexisting hypertension;

Gestational hypertension: hypertension first diagnosed after 20 weeks of gestation, not accompanied by proteinuria. If blood pressure returns to normal by 12 weeks post-partum it is called transient hypertension, otherwise it is considered as chronic hypertension.

Besides increasing the risk of maternal mortality and morbidity, most fetal adverse events (*i.e.*, intra-uterine grow restriction, preterm birth, low birth weight, and perinatal death) are attributable directly to preeclampsia [4]. In addition, recent studies have suggested that preeclamptic women [5–7] and their offspring [8] are at increased risk of cardiovascular and renal diseases later in life. Although its pathophysiology is not entirely known, there are several recognized risk factors for the development of preeclampsia, such as primiparity, multiple gestation, ethnicity, preexisting medical conditions (hypertension, diabetes), and obesity [9–11]. A higher incidence of these risk factors in industrialized countries over the last decades is a most likely cause for an increased rate of preeclampsia [1]. While the only definitive cure for preeclampsia is still delivery of the fetus and placenta [12], unraveling the etiologic mechanisms of preeclampsia may provide better approaches for treatment and ultimately prevention [13]. Therefore, the overall goal of this review is to provide an overview of the pathophisiology of preeclampsia, more specifically regarding the role of matrix metalloproteinases (MMPs), and the potential of MMP inhibitors as therapeutic options.

## 2. CARDIOVASCULAR ADAPTATIONS DURING NORMAL PREGNANCY

Several functional and anatomical alterations occur in the cardiovascular system of the pregnant woman to ensure nutrient supply to the fetus. During normal gestation, there is an expansion of 40–50% in blood volume [14–16], due to a greater increase in plasma volume than an increase in red blood cell mass, resulting in the physiologic anemia of pregnancy [17, 18]. An elevation in heart rate and stroke volume leads to an increase of 30–50% in cardiac output [14, 15, 19]. Additionally, there is a widespread vasodilatation, with increased arterial compliance and reduced peripheral vascular resistance [14, 15, 19], enhancing the blood flow especially in the uteroplacental circulation [20–22]. There is also an increase in renal blood flow (RBF) by 60–80% and in glomerular filtration rate (GFR) up to 50% [14, 23]. As a result of systemic vasodilatation and renal hyperfiltration, there is a decrease in systolic and diastolic blood pressure of about 5–10 mmHg [14, 15, 19, 24]. Moreover, these physiological alterations in preload and afterload enlarge the cardiac chambers, particularly the left ventricle which undergoes remodeling with increased wall thickness and mass [19, 25, 26]. Interestingly, this hemodynamic shift begins prior to placentation, reaches a peak in the second trimester of pregnancy, and then remains relatively constant until delivery [14, 15].

The cardiovascular adaptations to gestation are mainly induced by humoral and neural mechanisms, with multiple receptors and effectors interacting to regulate blood pressure [27]. Placental hormones, such as estrogen, progesterone and human chorionic gonadotropin, also exert a significant effect on maternal hemodynamics [28]. These hormones interact with the the renin-angiotensin-aldosterone system (RAAS) [29] and the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway to control blood pressure during pregnancy [30]. Studies in humans and pregnant models have implicated NO [31, 32] and prostacyclin [33, 34] as key vasodilators responsible for the reduced vascular resistance seen in pregnancy [35]. Another important mechanism contributing for the systemic vasodilatation is a decreased vascular responsiveness to angiotensin II [36].

These changes initiate further baroreceptor-mediated neurohormonal events, including activation of the RAAS [14, 37–39], with a subsequent increase in body sodium and water retention. Alterations in cortisol, vasopressin, kallikreins, vascular endothelial growth factor (VEGF), atrial natriuretic peptide, and in the sympathetic nervous system may also mediate cardiovascular adaptations during pregnancy [27, 35]. In addition, relaxin, an ovarian hormone secreted in large amounts by the placenta and decidua during gestation, appears to be an upstream mediator of the increased renal NO synthesis, resulting in an elevated RBF and GFR [40]. However, preeclamptic pregnancies are not accompanied by many of these alterations, being characterized by high vascular resistance, low plasma volume and reduced cardiac output [41–43], which ultimately leads to increased blood pressure in order to guarantee placental and fetal demands for oxygen and nutrients.

## 3. PATHOPHYSIOLOGY OF PREECLAMPSIA

Experimental studies in animals and humans have implicated placental ischemia and hypoxia as a central causative factor in the etiology of preeclampsia [44–46]. The pathophysiology of preeclampsia is thought to occur in two stages. The first stage is a poorly understood abnormality in the normal placentation process which is maternally asymptomatic. The second symptomatic stage is associated with altered proangiogenic and antiangiogenic factor balance, increased maternal oxidative stress, and immunological dysfunction. There is also widespread activation/dysfunction of the maternal vascular endothelium which results in enhanced formation of endothelin and decreased nitric oxide synthesis and/or bioavailability. These endothelial abnormalities, in turn, cause hypertension by impairing renal function and increasing total peripheral resistance. Recent research into both of these stages has revealed glimpses into the underlying origins of the disease and the mechanisms of the resulting maternal symptoms.

There is now growing awareness that immunological dysfunction is an important factor in the pathogenesis of preeclampsia [47]. While it is well established that pregnancy alone sets off an increased maternal inflammatory response, the secretion of inflammatory cytokines in preeclamptic women is markedly increased. Recent work has demonstrated that the production of tumor Necrosis Factor-α (TNF-α) and other inflammatory cytokines is primed by circulating syncytiotrophoblast microparticles, which are elevated in preeclamptic women [48]. In the reduced uterine perfusion pressure (RUPP) animal model of preeclampsia, circulating levels of interleukin-6 (IL-6) and TNF-α are elevated, and these alterations are consistent with those found in human subjects [49, 50]. Furthermore, infusion of IL-6 or TNF-α to levels consistent with those seen in preeclamptic women leads to gestational hypertension in rats [51]. Moreover, TNF-α blockade by the soluble TNF-α receptor etanerecept in the RUPP model partially attenuates the associated hypertension [51].

Another interesting recent development in our understanding of the role of immunity in the pathophysiology of preeclampsia is the identification of a circulating angiotensin II type-I receptor agonistic autoantibody (AT1-AA) [52–54]. The AT1-AA was found in the circulation of preeclamptic patients, and its epitope eventually mapped to the second extracellular loop of the AT1 receptor [54]. In response to placental ischemia in pregnant rats AT1-AA is produced to levels comparable to those seen in preeclamptic women [53]. Furthermore, chronic administration of the purified autoantibody to normal pregnant rats to levels seen in pregnant women and RUPP rats resulted in concurrent ~20% increases in blood pressure and dramatic increases in tissue expression of ET-1 [52].

Another promising area of research on the etiology of preeclampsia is the interplay between pro- and anti-angiogenic factors [55–58]. Soluble fms-like tyrosine kinase-1 (sFlt-1) is a

splice variant of the longer VEGFR-1 cell surface receptor in which the cytoplasmic and transmembrane domains have been post-transcriptionally excised. This molecule, which is produced by placental trophoblasts in its soluble form (sFlt-1), acts as an antagonist for the proangiogenic proteins VEGF and PlGF by sequestering free protein in the plasma, making them unavailable for receptor binding. Furthermore, elevated levels of sFlt-1 in both placenta and plasma were shown in preeclamptic women when compared to women with normal pregnancy [57].

Observations from a number of animal models of preeclampsia also implicate sFlt-1 as an important factor in the pathology of the disorder. Adenovirus vector expression of sFlt-1 in pregnant rats produced a preeclampsia-like phenotype, with increased arterial pressure, glomerular endotheliosis, and proteinuria [57]. Furthermore, placental ischemia induced by reductions in uterine perfusion pressure in rats resulted in increased plasma and placental sFlt-1 concentrations, results confirmed in a non-human primate model of placental ischemia [59, 60]. Chronic infusion of sFlt-1 into rats to circulating levels mimicking those seen in preeclamptic women leads to significant increases in maternal blood pressure, with concomitant decreases in fetal weight and increases in both placental and vascular reactive oxygen species, an important factor in endothelial dysfunction [61, 62].

Human studies indicate that the decidua in preeclampitic women has significantly higher levels of lipid hydroperoxides and free isoprostane, a byproduct of free-radical peroxidation of arachodonic acid. A number of oxidative stress markers have also been reported systemically in preeclamptic women, among these peroxynitrite [63–65]. In the placental ischemia rat (RUPP model) there is also an increase in oxidative stress during gestation, suggesting a link between placental ischemia/hypoxia and the production of reactive oxygen species [66]. The administration of a SOD mimetic drug (tempol) attenuated the hypertensive responses to placental ischemia [66]. In a related study, administration of the NADPH oxidase inhibitor apocynin also significantly attenuated placental ischemia-induced hypertension, suggesting this enzyme as an important player in the pathogenesis of preeclampsia in the RUPP animal model [63–66].

One promising target in the study of preeclampsia pursued by our group in recent years is the potent vasoconstrictor peptide endothelin [67]. The majority of clinical studies which have investigated endothelin showed elevated levels of this peptide in preeclamptic women when compared to healthy controls [68], although this difference has not been universally observed [69]. Interestingly, the hypertension induced by placental ischemia or TNF-α infusion in pregnant rats are completely abrogated by pretreatment with an endothelin receptor type A (ET-A) antagonist [67]. In addition, studies utilizing the AT1-AA infusion in a rat model of preeclampsia demonstrated elevated tissue endothelin production [67]. Administration of ET-A specific antagonists blunted the hypertension associated with this model [67]. Finally, increased cortical endothelin transcription was found in the animal model of preeclampsia induced by sFlt-1 infusion, and the ET-A receptor blockade normalized blood pressure [60]. The elevation in endothelin concentrations in response to these varied models of preeclampsia argues for an important role of this peptide in the pathophysiology of preeclampsia.

## 4. ROLE OF MMPS IN THE PATHOGENESIS OF PREECLAMPSIA

Trophoblasts are important precursor cells from the human placenta which exert critical roles to promote a healthy gestation including embryo implantation, hormone production, fetal immune protection, and placental vascularization. In the first trimester of a normal pregnancy, cytotrophoblastic cells invade the uterine tissue and migrate against the bloodstream into the maternal spiral arteries, where they undergo differentiation into cells

with endothelial phenotype. The trophoblastic invasion of maternal vessels results in extracellular matrix remodeling, which gives rise to high uteroplacental vessel distensibility to accommodate the increased blood flow [70–72]. In preeclampsia, however, trophoblastic invasion is reduced, leading to incomplete modification of maternal spiral arteries and therefore to decreases in placental perfusion [46, 73–75].

A prerequisite for the trophoblastic invasion success, angiogenesis and embryogenesis is the degradation and remodeling of the uterine extracellular matrix (ECM) [76, 77]. Remodeling of the umbilical cord vessels may also contribute to decreased blood flow to the fetus of women with preeclampsia.

Matrix metalloproteinases (MMPs) are a family of structurally related, zinc-dependent enzymes with multiple functions and tissue distribution [78]. Their activity target extracellular matrix components during development and morphogenesis. Specifically, MMP-2 and MMP-9 are involved in remodeling of placental and uterine arteries [79, 80], and abnormal expression of these MMPs has been reported in hypertensive disorders of pregnancy. Indeed, there is now evidence that MMPs may affect the vascular function and play a role in the vascular alterations found in preeclampsia and in other cardiovascular diseases [81–86].

Under normal circumstances, MMP activity is regulated at the level of transcription, activation of latent forms, and inhibition by endogenous MMP inhibitors (tissue inhibitors of metalloproteinase; TIMPs) [87]. Interestingly, functional genetic polymorphisms apparently modify MMP-2 transcriptional levels and may contribute to disease conditions by affecting MMP-2 transcriptional levels [88]. While MMP-9 activity is regulated at different levels including activation of MMP-9 latent forms, by interaction with TIMPs, especially TIMP-1, it is also regulated at the transcriptional level [89]. Again, genetic polymorphisms in the MMP-9 gene were also shown to affect MMP-9 transcription [90] and disease susceptibility [91, 92].

The ischemic placenta releases vasopressors into the maternal circulation that modify endothelial function by altering the balance between vasodilators (nitric oxide, prostacyclin) and vasoconstrictors (endothelin-1, increased response to angiotensin II) [13, 93–95]. The endothelial dysfunction affects multiple maternal organs, and the impaired control of vascular function contributes to hypertension and increased glomerular vascular permeability, thus leading to proteinuria, an important feature of preeclampsia.

Although unproven in the particular context of preeclampsia, it is possible that upregulated MMP activity in preeclampsia promotes increased concentrations of vasoconstrictors including endothelin-1-related peptides, and reduced concentrations of vasodilators including adrenomedullin and calcitonin gene related-peptide, as previously suggested [96–99]. These previous studies suggest that imbalanced MMP activity apparently generates vasoconstrictors and degrades vasodilators, promoting vasoconstriction and hypertension [96–99]. In addition, the release of the proform of TNF-α from its membrane-bound site is an MMP-dependent process [100, 101]. Conversely, TNF-α indirectly stimulates the proteolytic activity of MMPs [102], especially during the implantation process [103, 104], suggesting that abnormal MMPs and inflammatory mediators may interact contributing to the features of this syndrome.

Moreover, endothelial dysfunction in preeclampsia may result of oxidative stress and reduced nitric oxide bioavailability [62–65, 82]. Indeed, increased concentrations of reactive oxygen species including superoxide may enhance vascular concentrations of peroxynitrite, a powerful oxidizing agent that contributes to the pathogenesis of many cardiovascular

including preeclampsia [64]. This agent may directly activate MMPs [84], although this mechanism has not been clearly shown in preeclampsia.

Activated MMPs may also contribute to cardiovascular dysfunction in preeclampsia through proteolysis of cell surface receptors, such as VEGFR-2 and β(2)-adrenergic receptor, as previously shown in other animal models of cardiovascular diseases (Fig. 1) [105–109]. However, these suggestions remain to be proved in preeclampsia.

## 5. CLINICAL FINDINGS SHOWING MMP ALTERATIONS IN PREECLAMPSIA

Although MMPs have an important function in tissue formation and remodeling during pregnancy, only few studies have evaluated the role of MMP-2 and MMP-9 in the pathophysiology of preeclampsia. Huisman *et al.* studied MMP-2 and MMP-9 in placental bed biopsies as early as 10–12 weeks of gestation, but their levels were not different when uncomplicated pregnancies were compared with pregnancies complicated by preeclampsia/HELLP syndrome [110]. These findings are not in agreement those reported by Kolben *et al.* [111] and Shokry *et al.* [112], who observed reduced immunologically defined MMP-9 levels in preeclamptic placental tissues collected at delivery. In addition, Galewska *et al.* used distinct techniques to show that preeclamptic umbilical cord tissues (artery and vein) had lower MMP-2 and MMP-9 levels than healthy tissues [113]. However, they found increased MMP-9 levels in plasma obtained from umbilical cord blood samples from preeclamptic newborns, and no significant differences in MMP-2, TIMP-1 and TIMP-2 compared with those measured in healthy pregnancies [114]. Furthermore, Lavee *et al.* demonstrated increased MMP-2 (by ELISA) and TIMP-2 (by western blotting) levels in aminiotic fluid of women who subsequently develop preeclampsia [115]. Interestingly, they determined pro-MMP-9 levels in normal amniotic fluid, but zymogram wells loaded with preeclamptic amniotic fluid did not present any MMP-9 bands [115].

Regarding circulating MMP-9 and TIMP-1, Kolben *et al.* found no significant differences in immunoreactive plasma MMP-9 concentrations between preeclamptic/eclamptic patients and healthy pregnant women [111]. While we have used zymography to show no differences in pro-MMP-9 levels [116], ELISA assays revealed that plasma MMP-9 concentrations may be increased in preeclampsia [117]. Moreover, we found elevated plasma TIMP-1 concentrations in preeclampsia, but no differences in MMP-9/TIMP-1 ratios [116, 117]. Montagnana *et al.* also observed no differences in serum immunoreactive MMP-9 and increased TIMP-1 concentrations in preeclampsia [118]. Conversely, Myers *et al.* found that western blotting defined plasma TIMP-1 levels were not altered in preeclampsia, although they reported decreased levels in the same patients before diagnosis [119]. Interestingly, Poon *et al.* showed that plasma MMP-9 concentrations are increased in women prior to presentation of preeclampsia. However, this biomarker did not contribute significantly to prediction of disease [120].

With respect to circulating MMP-2 and TIMP-2 levels, Davidge's group has used zymographic techniques to show higher plasma MMP-2 levels in preeclamptic patients [121] and in women who subsequently develop preeclampsia [119] compared to healthy pregnant. Although plasma pro-MMP-2 (by zimography) and TIMP-2 levels were not statistically different in our small cohort with 32 weeks of gestation [116], MMP-2 (by ELISA) and TIMP-2 concentrations were elevated in preeclampsia when we studied a larger number of patients at 36 weeks of gestation [122]. None of our studies revealed differences in MMP-2/TIMP-2 ratios [116, 122]. Additionally, Montagnana *et al.* observed increased serum immunoreactive MMP-2 concentrations, but no significant differences in TIMP-2, in preeclamptic patients compared with healthy pregnant women [118].

Genetic reports have also been divergent regarding the association of MMP-2 and MMP-9 polymorphisms with preeclampsia. Coolman *et al.* observed a reduced prevalence of the rare T allele of the MMP-9 $C^{-1562}T$ polymorphism in preeclampsia [123]. Intriguingly, *in vitro* studies have showed that the "C" to "T" substitution at −1562 position of the MMP-9 promoter increases MMP-9 gene expression [90]. Therefore, the lower frequency of the 1562T allele in preeclamptic patients suggests that they may have decreased MMP-9 levels, which might predispose them to maladaptation of the spiral arteries and decreased degradation of the decidua. However, MMP-9 polymorphisms were not linked to preeclampsia in Fraser *et al.* [124] and our studies [117, 125]. In addition, we did not find significant associations between MMP-2 polymorphisms and preeclampsia [122, 126], although genetic variations of the $C^{-1306}T$ and $C^{-735}T$ polymorphisms were associated with altered plasma MMP-2 and TIMP-2 concentrations in preeclamptic patients [122]. Taken together, these findings suggest that altered MMPs and TIMPs levels may contribute to preeclampsia. However, further studies are warranted to establish how imbalanced MMP activity may contribute to the pathogenesis of preeclampsia.

## 6. EVIDENCE SUPPORTING THE USE OF MMP INHIBITORS IN PREECLAMPSIA

Although antihypertensive drugs do not reverse the pathogenic processes in preeclampsia, they are used to prevent and treat severe hypertension, and to extend pregnancy for as long as possible. A major challenging issue is to decide what blood pressure levels should be targeted to minimize maternal and neonatal complications and avoid fetal distress and toxicity [127]. Currently, options of anti-hypertensive drugs for preeclampsia are limited, and only few drugs have been adequately evaluated in pregnant women. Methyldopa is the drug of choice based on its well documented first trimester safety and long follow-up in neonates. Second-line agents include nifedipine, hydralazine and labetalol, which are commonly used when monotherapy with methyldopa is insufficient or in cases of methyldopa intolerance [3, 12, 127, 128].

As highlighted previously, many complex mechanisms mediate the widespread endothelial dysfunction seen in preeclampsia, leading to diverse clinical features, such as hypertension, proteinuria, edema, and cerebral and hepatic disturbances. The key role of MMPs in these cardiovascular alterations has been demonstrated in different animal models of hypertension [129–132]. Collectively, these reports have found increased MMP-2 or MMP-9 activity in different tissues, and treatment with doxycycline (a nonspecific MMP inhibitor) ameliorated hypertension, vascular dysfunction and artery/cardiac remodeling associated with this condition. Moreover, another broad-spectrum MMP inhibitor markedly blunted the age-associated increases in arterial pressure via retardation of age-associated proinflammatory signaling, preservation of intact elastin fibers, and reduction of collagen deposition [133].

To our knowledge, only one study has tested MMP inhibitors in experimental models of preeclampsia [134]. Verlohren *et al.* showed that doxycycline treatment from gestational day 12 to 18 resulted in lighter placentas and intrauterine growth restriction in both control and preeclamptic pregnant rats. Additionally, doxycycline reduced trophoblastic invasion and placental perfusion only in the preeclamptic group. However, they did not evaluate the effect of doxycycline on the development of pregnancy-induced hypertension [134]. Although, previous studies have also reported adverse effects of MMP inhibitors on pregnant animals [135–138], such effects were not reported by others [139–141]. Indeed, doxycycline is the only MMP inhibitor currently approved by the U.S. Food and Drug Administration (FDA) [142] and, when prescribed as an antibiotic during pregnancy, it is classified as a potentially teratogenic medication (class D: potential benefits from the use of the drug in pregnant women may be acceptable despite its potential risks) [143]. However, human studies have

led to the conclusion that the teratogenic risk of doxycycline is unlikely [144–146]. Moreover, since it is used as an MMP inhibitor at sub-antimicrobial doses [147], we would expect that doxycycline causes minimal or no fetal adverse reactions when used at this dosage. Nevertheless, its use should be carefully monitored during pregnancy.

It is now clear that some antihypertensive drugs may have important effects on circulating MMP concentrations [148]. Among the drugs usually prescribed to patients with preeclampsia, clinical studies have evaluated only the effect of calcium channel blockers on MMP levels [149–151]. For example, lercanidipine therapy for 15 days decreased plasma MMP-9 activity, whereas MMP-2 and TIMP-1 levels remained unaltered [151]. Conversely, treatment of hypertensive patients with amlodipine for 6 months increased plasma MMP-9 levels back to the concentrations seen in controls [150]. Treatment with felodipine increased plasma MMP-2 concentrations, whereas diltiazem had no effects on circulating MMPs [149]. In addition, studies in rodent hypertensive models suggest similar effects of different calcium channel blockers (lercanidipine, nifedipine, nimodipine, and amlodipine) on vascular MMP-2 activity, which inhibited cardiovascular remodeling [152–154].

Since some antihypertensive drugs affect cardiovascular MMP activity, we examined whether circulating MMP-2 and MMP-9 levels are different in preeclamptic women who respond to antihypertensive therapy as compared with those who do not respond to therapy. We found lower plasma MMP-9 concentrations and MMP-9/TIMP-1 ratio (an index of net MMP-9 activity) in non-responsive patients compared to responsive patients, thus suggesting that the most severe cases of the disease may have undergone abnormal remodeling of placental and uterine tissues [117]. Conversely, we observed higher plasma MMP-2 concentrations and MMP-2/TIMP-2 ratio (an index of net MMP-2 activity) in non-responsive patients compared to responsive patients [126], thus suggesting that antihypertensive drugs may ameliorate preeclamptic symptoms by decreasing net MMP-2 activity.

We have also examined whether MMP-2 and MMP-9 polymorphisms affect the responses to antihypertensive therapy in preeclampsia. While a MMP-9 haplotype (the combination of T and H alleles of the $C^{-1562}T$ and $-90(CA)_{13\text{-}25}$ polymorphisms, respectively) was associated with lack of responsiveness [117], MMP-2 polymorphisms were not linked to variability in responsiveness [126]. In view of these findings, we might speculate that preeclamptic patients would benefit from the use of MMPs inhibitors, at least during the systemic phase of the disease.

Finally, low-dose aspirin has been suggested as a pharmacological treatment to prevent the development of preeclampsia [155, 156] especially in high risk patients [157–159]. Since, aspirin may also affect MMPs [160–163], the association of doxycycline with aspirin may have synergic action in this syndrome. However, despite the growing evidence indicating that MMPs are pharmacological targets in cardiovascular diseases [142, 164, 165], it remains to be determined whether the use of MMPs inhibitors would improve maternal and fetal outcomes in preeclampsia.

## CONCLUSIONS AND FUTURE PERSPECTIVES

The primary event in preeclampsia is proposed to be poor placental perfusion which leads to widespread maternal endothelial dysfunction. However, the causes of the reduction in the placental blood flow have yet to be fully elucidated. It has been suggested that decreased placental MMP expression may cause shallow cytotrophoblastic invasion and incomplete remodeling of the spiral arteries. MMPs are also thought to act by linking placental ischemia and cardiovascular dysfunction. Supporting a potential role of MMPs in preeclampsia are findings that placental and amniotic liquid MMP-9 levels are decreased, and plasma

concentrations of MMP-2 and MMP-9 are elevated in preeclamptic women, even before the appearance of clinical symptoms. MMP-2 can stimulate vasoconstriction through the cleavage of different peptides. Moreover, MMP-9 cleaves surface receptors involved in angiogenesis and vasodilatation processes. Nevertheless, MMP-9 haplotypes have been associated with lack of responsiveness to antihypertensive drugs in preeclampsia. Collectively, these findings suggest that MMP-2 and MMP-9 may play a role in causing hypertension during pregnancy through multiple complex pathways.

The determination of plasma MMP-2 and MMP-9 concentrations and genotypes for MMP-9 polymorphisms may be valuable tools to predict which patients are at increased risk of developing preeclampsia, and which will respond to antihypertensive therapy, respectively. Such patients could benefit from the use of MMPs inhibitors such as doxycycline. However, the quantitative importance of MMPs in mediating high blood pressure and other features of preeclampsia remains unclear, and new basic and clinical studies are required, especially those designed to examine MMP inhibitors as adjuvant therapy of preeclampsia.

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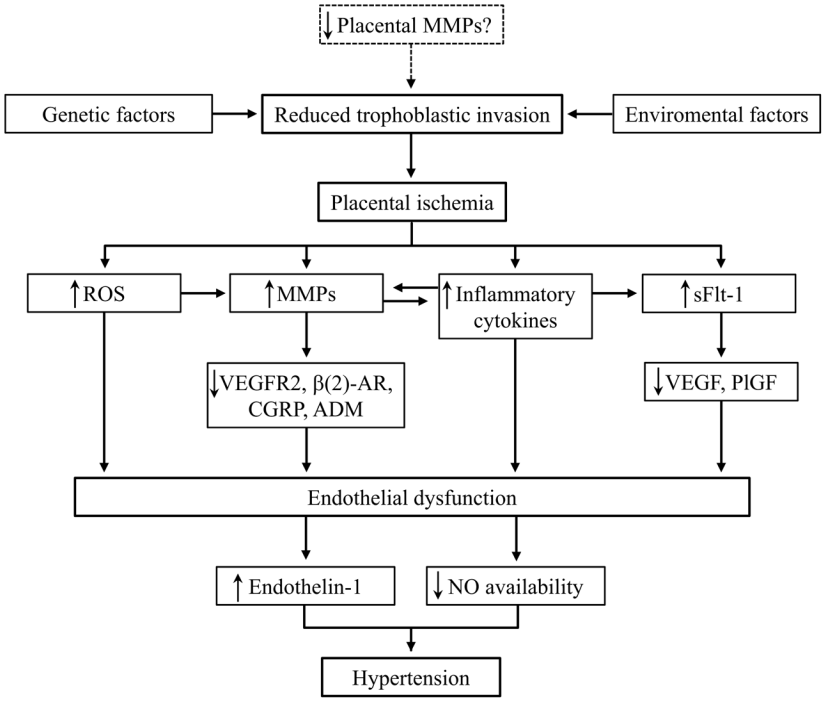


**Fig. 1.**

Possible involvement of MMPs in the pathogenesis of preeclampsia. MMP inhibitors such as doxycycline may prevent the alterations associated with imbalanced MMP activity in preeclampsia. MMP, matrix metalloproteinase; ROS, reactive oxygen species; sFlt-1, soluble fms-like tyrosine kinase-1; VEGFR2, vascular endothelial growth factor receptor-2; β(2)-AR, β(2)-adrenergic receptor; CGRP, calcitonin gene related-peptide; ADM, adrenomedullin; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; NO, nitric oxide.