

**Matrix metalloproteinases: control of vascular function and their potential role in preeclampsia**

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**1. ABSTRACT**

Preeclampsia is a pregnancy specific disorder characterised by hypertension and proteinuria occurring after the twentieth week of gestation. Preeclampsia induced hypertension is the result of increased vascular reactivity and endothelial dysfunction, however, the mechanisms underlying this state remain elusive. One possible mediator may be the matrix metalloproteinases (MMPs), a family of proteinases typically recognized for long term tissue remodelling. This review examines the evidence suggesting a role for MMPs in acutely regulating vascular function. Studies have shown that MMPs can activate vasoconstrictors (e.g. endothelin), inactivate vasodilators (e.g. calcitonin gene related peptide) and transactivate cell surface receptors responsible for vasoconstriction (e.g. epidermal growth factor receptor). The potential role of these proteinases in preeclampsia will then be discussed.

**2. PREECLAMPSIA – AN INTRODUCTION**

Preeclampsia is a multisystem disorder that is exclusive to human pregnancy. This condition is defined by blood pressure over 140/90 mmHg and proteinuria ( $\geq 0.3$  g in a 24 hour urine sample) after 20 weeks of gestation (1). In spite of the advances in prenatal care and extensive research in the area, preeclampsia still complicates 4-5% of all pregnancies and remains one of the leading causes of maternal and fetal morbidity and mortality (2). In addition, preeclampsia is associated with maternal complications such as acute renal failure, cerebral hemorrhage, pulmonary edema, the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), and eclampsia when seizures develop (3). Interestingly, women with preeclampsia also have a higher risk of cardiovascular morbidity and mortality later in life (4).

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Although it is known that factors such as obesity (5), family history of heart disease (6), diabetes (7), and hypertension (8) increase the risk of preeclampsia, there is no effective prevention or treatment of this condition except delivery of the placenta. Moreover, the cause and pathophysiology of the syndrome remain unclear despite intensive study. A current theory argues that preeclampsia is a two stage disorder (9). In the first stage, reduced placental perfusion caused by poor remodeling leads to the release of circulating factors, including cytokines and syncytiotrophoblast microparticles. These factors then enter the maternal circulation in the second stage and cause a vascular dysfunction that is characterized by endothelial dysfunction and increased vasoreactivity to pressor agents (10). This vascular dysfunction ultimately results in the clinical manifestations of preeclampsia. To date, however, the linkage between the two stages has not been elucidated.

One mediator of vascular tone in preeclampsia may be the family of proteinases known as matrix metalloproteinases (MMPs). MMPs are zinc-dependent proteinases that have long been recognized to contribute to long term remodeling processes such as embryogenesis and atherosclerosis (11). They achieve this through their ability to cleave the extracellular matrix. More recently, MMPs have also been shown to play a role in acute processes not involving extracellular matrix degradation, such as platelet aggregation (12), septic shock (13), and ischemia-reperfusion injury of the heart (14). Of particular interest for the study of preeclampsia is the growing body of evidence that MMPs can acutely cause vasoconstriction and regulate vascular function. This review provides a basic introduction to MMPs and focuses on their role in the regulation of vascular tone (summarized in Figure 1). Evidence linking MMPs to preeclampsia will also be detailed.

### 3. MATRIX METALLOPROTEINASES – AN OVERVIEW

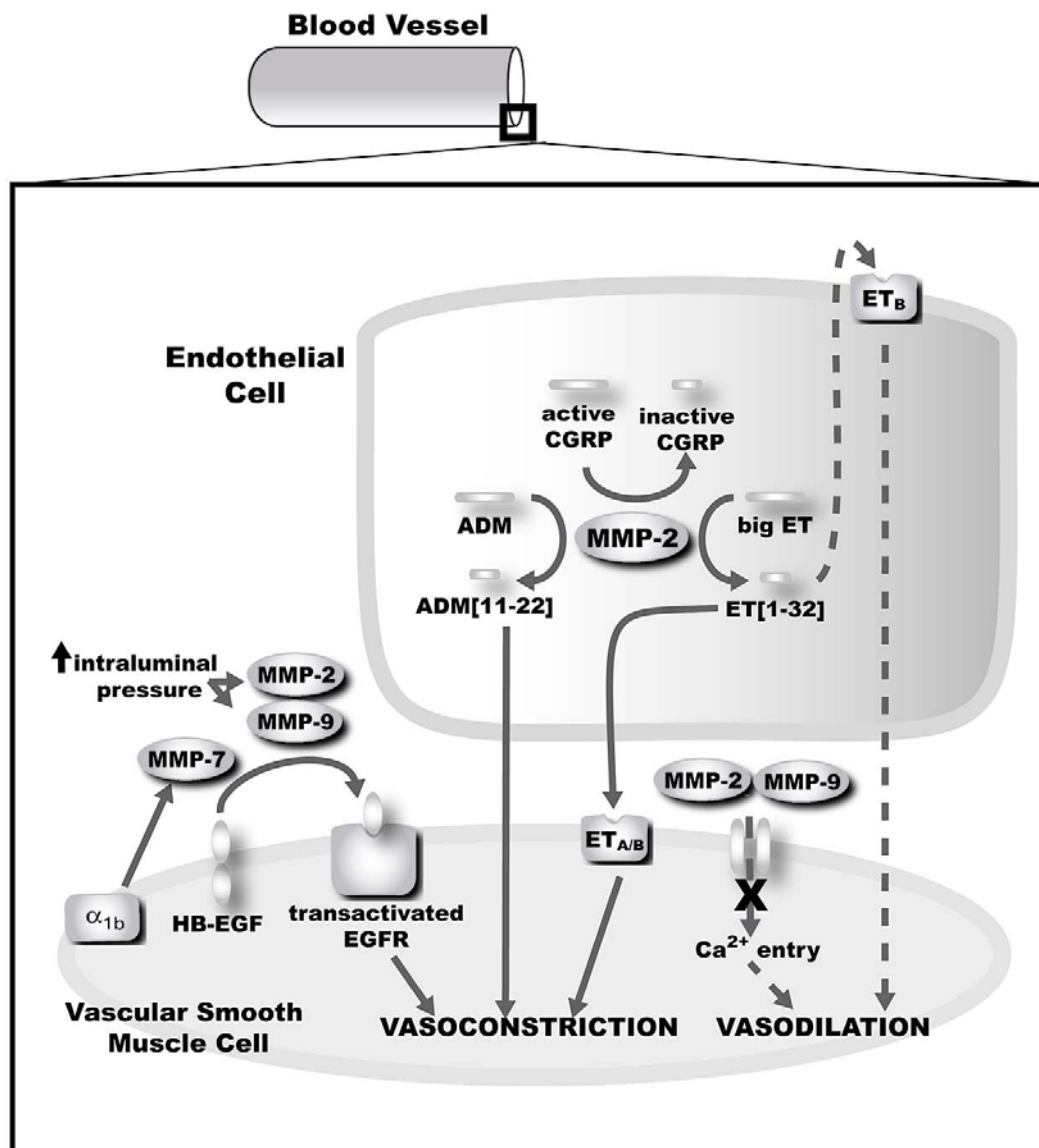
The study of MMPs began with the seminal studies of Gross and Lapiere in 1962 (15). While investigating the mechanisms underlying resorption of the tadpole tail they isolated an enzyme capable of degrading collagen molecules into  $\frac{3}{4}$  and  $\frac{1}{4}$  length fragments. This enzyme was subsequently labelled collagenase 1 (MMP-1). Over the course of the last forty-five years a number of other MMPs have been identified and this large family of proteins now includes over twenty mammalian members that have been identified in a variety of cell types (16). They are classified by numerical designation (MMP-1 through MMP-28) and also according to their *in vitro* substrate specificity. A number of different classes have been described: collagenases (MMPs -1, -8, and -13), membrane-type MMPs (MT-MMPs, 1 through 8), and gelatinases (MMP-2 and -9). Four endogenous tissue inhibitors of MMPs (TIMPs 1 through 4) have also been identified [reviewed by Nagase and Visse (17, 18)]. It is now believed that an imbalance between MMPs and TIMPs produces excessive proteolytic activity that may contribute to a number of pathological states.

The overall domain structure of all known MMPs is very similar (Figure 2). At the N-terminus, most MMPs have a signal peptide that allows for secretion and transport out of the cell. Next to this signal peptide, is a hydrophobic propeptide domain that shields the catalytic domain next to it; thus, since all MMPs possess this propeptide domain, all MMPs are inactive zymogens when they are synthesized. Finally, at the C-terminus most MMPs also have a hinge region that connects to a hemopexin domain. This hemopexin domain confers substrate specificity and coordinates docking with other proteins. The catalytic domain of all MMPs is a highly folded region that contains the “zinc binding consensus sequence”. This region must coordinate with a catalytic zinc ion, a structural zinc ion, and two to three structural calcium ions (19). In its zymogen form, the catalytic domain coordinates to a cysteinyl sulphhydryl group on the propeptide domain [also called the ‘cysteine switch’(20)] and is rendered inactive.

MMP activity is regulated by their endogenous inhibitors, the TIMPs. All four TIMPs have been demonstrated to inhibit almost all known MMPs (18). The TIMPs are “wedge” shaped proteins that inhibit MMPs by locking into the catalytic domain (21). The dysregulation of TIMPs appears to be as significant pathologically as the dysregulation of MMPs. For instance, TIMP-3 gene deletion results in heart failure (22), and overexpression of TIMP-2 can prevent atherosclerotic plaque progression in animal models (23).

MMPs may undergo one of four different processes in order to become activated: a) activation in the extracellular space, b) activation at the cell surface by MT-MMPs, c) intracellular activation, or d) activation by oxidative stress. The first two mechanisms have been well characterized by a variety of groups [reviewed in (24)] and involve cleavage of the propeptide domain by either membrane-type MMPs at the cell surface (25) or another proteinase extracellularly [e.g. plasmin (26)]. Intracellular activation by furin-like proprotein convertases has been shown to occur for a number of MMPs (27, 28). After intracellular activation has occurred, the active MMP is shuttled either to the cell membrane for insertion (in the case of MT-MMPs) or secretion.

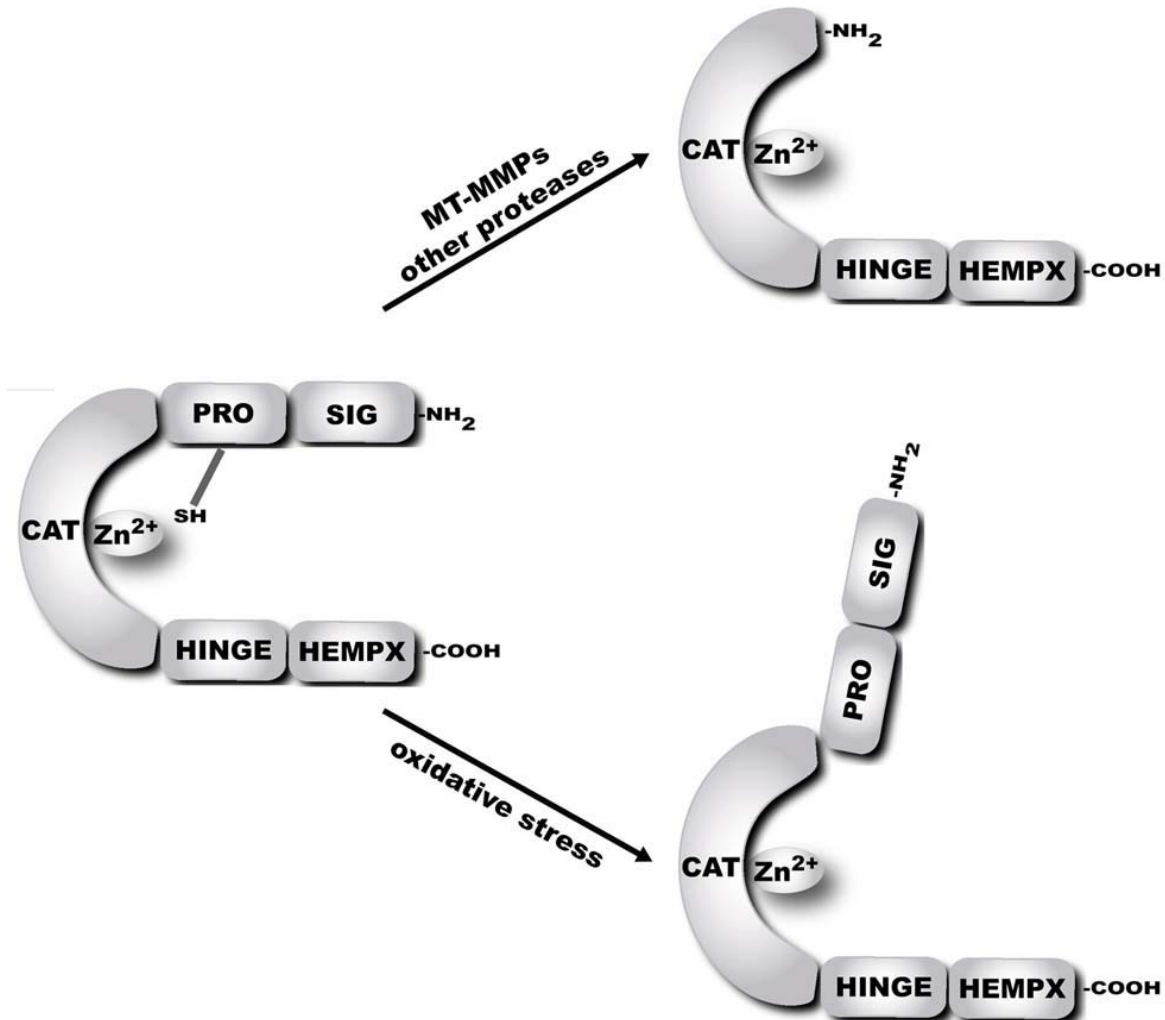
Activation of MMPs by oxidative stress (29-32) is an emerging concept that may be of particular relevance in preeclampsia since this condition has been associated with increased peroxynitrite production (the destructive reaction product of superoxide anion and nitric oxide) (33, 34). Peroxynitrite can react with the sulphhydryl bond of the cysteinyl group that binds the catalytic  $Zn^{2+}$  and thus expose the catalytic domain [details of this reaction can be found in (30)]. Unlike other activation mechanisms, oxidative activation produces an ‘activated zymogen’ since the propeptide domain is not removed (Figure 2). Nitric oxide alone may also affect MMP activity, however, many studies have demonstrated both negative and positive regulation of MMPs by nitric oxide (35, 36). The variability in these observations is likely a result of the different models and pathologies studied.



**Figure 1.** Overview of matrix metalloproteinase regulation of vascular tone. Matrix metalloproteinases (MMPs) promote vasoconstriction and vasodilation through several pathways. *Vasoconstriction:* MMP-2 can a) cleave and inactivate the vasodilator calcitonin gene related peptide (CGRP); b) cleave the vasodilator adrenomedullin (ADM) [11-22]; c) cleave big endothelin (big ET) to form the vasoconstrictor endothelin (ET) [1-32] which binds to smooth muscle endothelin A or B receptors (ET<sub>A/B</sub>). An increase in intraluminal pressure can increase MMP-2 and MMP-9 activity, and both of these MMPs can cleave smooth muscle cell bound heparin-binding epidermal growth factor (HB-EGF). Cleaved HB-EGF subsequently transactivates the epidermal growth factor receptor (EGFR) and causes vasoconstriction. Similarly, when adrenoceptors ( $\alpha_{1b}$ ) are stimulated there is an increase in MMP-7 activity. MMP-7 can also cleave HB-EGF and transactivate the EGF-receptor. *Vasodilation:* ET[1-32], formed by MMP-2, can bind to ET<sub>B</sub> receptors on the endothelium, which subsequently promotes vasodilation. MMP-2 and MMP-9 can also inhibit Ca<sup>2+</sup> entry into smooth muscle cells. Potential roles for MMPs in preeclampsia induced vascular dysfunction remain to be elucidated.

Another emerging concept is the recognition that these proteinases act on non-matrix substrates (37). Typically, MMPs were acknowledged to contribute to long-term processes through tissue remodeling. Recently, however, MMPs have also been shown to contribute to more acute processes through the cleavage of non-matrix substrates. For instance, MMPs may modulate

inflammation by processing cytokines such interleukin-1 $\beta$ , monocyte chemoattractant protein-1, and tumor necrosis factor- $\alpha$  (38-40). This modulatory ability may also be of importance in preeclampsia, since this condition is characterized as an inflammatory state. MMPs can also cleave a number of proteins involved in vascular regulation. The remainder of this review focuses on the



**Figure 2.** Domain structure and activation of matrix metalloproteinases. Most matrix metalloproteinases have a signal peptide (SIG), a propeptide domain (PRO), a catalytic domain (CAT) that houses the catalytic zinc molecule (Zn<sup>2+</sup>), a hinge region, and a hemopexin domain (HEMPX). In its zymogen form the propeptide shields the catalytic zinc via a cysteinyl sulphhydryl group (-SH). MMPs can be activated by membrane-type MMPs (MT-MMPs) or other proteases (top arrow, see text for details). This results in exposure of the catalytic domain through proteolytic removal of the propeptide domain. MMPs can also be activated by oxidative stress (bottom arrow). This results in disruption of the cysteinyl sulphhydryl group that shields catalytic zinc, and produces an active MMP without removal of the propeptide domain.

putative role of MMPs in regulating vascular tone. Three MMPs in particular have been shown to regulate vascular tone, MMP-2, MMP-9, and MMP-7.

#### 4. MATRIX METALLOPROTEINASES AND VASCULAR REGULATION

##### 4.1. MMP-2 and MMP-9

MMP-2 (gelatinase A) was first described by Sellers et al in 1978 when they were able to separate its gelatinolytic activity from other MMPs (41). MMP-2 has subsequently become the most studied MMP with over 6000 references detailing its involvement in a variety of conditions. MMP-9 was first detected in 1972 by Harris and Kane in synovial fluid (42). MMP-9, like MMP-2, has

an *in vitro* substrate preference to gelatin, and is thus also labelled gelatinase B. Structurally, the gelatinases contain all the domains previously described, as well as a triple repeat fibronectin type II domain that confers their ability to bind to gelatin substrates (43).

The functional significance of MMP-2 in vascular regulation was first described through its effects on the vasoconstrictor endothelin-1 (ET-1) (44). ET-1[1-21] is usually formed through proteolytic cleavage of its precursor big endothelin-1[1-38]. This cleavage is mediated by the cell membrane associated endothelin-converting enzymes, a group of proteinases distinct from the MMPs (45). Our group found MMP-2 could also cleave big ET-1 to form a novel peptide ET-1[1-32] (Figure

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1). Using perfused resistance sized arteries from rats, it was demonstrated that this novel peptide was significantly more potent than either big ET-1 or ET-1[1-21]. Moreover, the vasoconstrictor effects of big ET-1 could be abolished by infusion of either the endogenous inhibitor TIMP-2, or a neutralizing antibody to MMP-2 (both of which presumably inhibited the conversion of big ET-1 to ET-1[1-32] by MMP-2 found in the artery wall). Interestingly, MMP-2 processing of big ET-1 occurred in endothelium denuded arteries. This suggests that: a) MMP-2 activity was present in the subendothelial layer, and b) MMP-2 may have a significant role in processing big ET-1 in pathological conditions like preeclampsia that are characterized by endothelial dysfunction/damage.

In a separate investigation, MMP-2 was also found to cleave and inactivate the vasodilator calcitonin gene-related peptide (CGRP) (46). Addition of CGRP to isolated arteries causes a concentration dependent relaxation, however, the addition of MMP-2 along with CGRP significantly decreased this vasodilatory effect. This was further supported by experiments with a highly selective gelatinase inhibitor ('CTT') (47) that demonstrated MMP-2 inhibition could induce vascular relaxation in a time and concentration dependent manner. When the arteries were pretreated with capsaicin, a drug that depletes endogenous CGRP stores, CTT no longer induced vascular relaxation.

Similarly, Martinez et al. found that MMP-2 could also cleave adrenomedullin, another vasodilator in the same family of proteins as CGRP (48). In this study, it was demonstrated that MMP-2 could process adrenomedullin to form a novel peptide vasoconstrictor (adrenomedullin [11-22]). Infusion of the peptide into normal rats produced a transient but significant rise in blood pressure. As well, this peptide could be found in the urine of normal human volunteers, suggesting that adrenomedullin[11-22] may have a physiological role. Interestingly, in the hypertensive state of preeclampsia there is a lack of compensatory elevation of both CGRP and adrenomedullin (49, 50). It remains to be determined whether MMP-2 contributes to these inappropriately low levels by degrading both of these vasodilators.

MMP-2 and MMP-9 have also been shown to regulate myogenic tone, an intrinsic property of arteries that allow them to contract in response to increased intraluminal pressure, and relax in the response to a reduction in pressure. This behaviour maintains constant blood flow and helps control peripheral resistance (51). In isolated arteries subjected to increasing intraluminal pressure, Lucchesi et al found that myogenic responses could be decreased partially by an MMP-2 inhibitor, and could be completely abolished by a combined MMP-2/-9 inhibitor (52). Teasing out the molecular mechanisms underlying this response, the investigators discovered that MMP-2 and -9 activities were increased in an intraluminal pressure-dependent manner, and that these MMPs cleaved cell membrane associated heparin binding-epidermal growth factor (HB-EGF) (Figure 1). This cleavage product could then transactivate the EGF-receptor, ultimately

producing vasoconstriction through intracellular signalling pathways [reviewed in (53)]. These results may be of particular relevance for preeclampsia, since myogenic tone is increased in models of preeclampsia when compared to normotensive pregnant groups (54, 55).

### 4.2. MMP-7

MMP-7, otherwise known as matrilysin, is the smallest member of the MMP family as it only contains the propeptide and catalytic domains. It was first purified and characterized from the rat uterus during involution postpartum (56). The role of MMP-7 in vasoconstriction was recently uncovered by Fernandez-Patron's group (57). Using isolated perfused rat arteries it was found that phenylephrine induced vasoconstriction increased MMP-7 activity. Similar to MMP-2 and -9 described above in the Lucchesi et al study, this MMP-7 activity cleaved HB-EGF and promoted transactivation of the EGF receptor (Figure 1). *In vivo*, MMP-7 activity was significantly greater in arteries taken from the spontaneously hypertensive rat, when compared to arteries taken from normotensive rats. When these hypertensive rats were given a broad spectrum MMP inhibitor their blood pressure was significantly reduced. In a follow up study, it was demonstrated that MMP-7 induced vasoconstriction through the production of reactive oxygen species from the mitochondria (58). This was a particularly significant finding since it suggested a connection between MMPs, the production of oxidative stress, and enhanced vascular tone, all of which are implicated in the development of hypertensive disorders like preeclampsia. To date, however, no study has directly examined the role of MMP-7 in preeclampsia, thus this may be an avenue of research that deserves further attention.

## 5. MATRIX METALLOPROTEINASES AND VASODILATION

Although the above studies support a vasoconstrictor role of MMPs, several investigations have also reported a possible vasodilatory role for MMPs. In models of septic shock, in which blood vessels are inappropriately vasodilated, MMP inhibition can increase vascular tone (59), and MMP deficiency can protect against endotoxic shock (13). In two independent studies, exogenous MMP-2 induced vasodilation in normal rat aortas (60) and in rat mesenteric arteries (61). In the former study, MMP-2 and MMP-9 were found to inhibit entry of  $Ca^{2+}$  into endothelium denuded aortic strips (Figure 1). In the latter study using mesenteric arteries, thrombin-induced vasorelaxation was mediated through release of MMP-2 as long as the endothelium was present.

In resistance sized arteries the apparent vasodilatory *and* vasoconstrictive effects of MMPs may be due to the presence of an intact, healthy endothelium. Specifically, MMP induced vasodilation may in fact be mediated by the formation of ET[1-21], which subsequently acts on endothelial  $ET_B$  receptors that mediate vasodilation (as opposed to vascular smooth muscle  $ET_{A/B}$  receptors that mediate vasoconstriction, Figure 1). Interestingly, Conrad's group found that relaxin-induced

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renal vasodilation in pregnant rats was mediated by the release of MMPs (62). Furthermore, the application of TIMP-2 actually promoted vasoconstriction in endothelium intact vessels, as opposed to the previously described vasodilatory effects of this MMP inhibitor on endothelium denuded vessels (44). In support of the theory that endothelin is cleaved by MMPs, Jeyabalan et al (62) also demonstrated that ET<sub>B</sub> receptor-deficient rats were resistant to relaxin induced vasorelaxation, despite an increase in MMP activity with relaxin infusion.

The Jeyabalan et al study was also significant since it was one of the few investigations of normal pregnancy induced changes of MMPs in the cardiovascular system. They demonstrated that in normotensive pregnant rats MMP-2 activity in vascular wall is significantly increased compared to virgin rats (62). In another study, Kelly et al determined that MMP-2 and MMP-3 are increased in the rat aorta during late gestation (63). A similar study by this group found that MMP-2, -3, -7, -9, -12, -13 and MT1-MMP mRNA were increased in the rat uterine artery during late gestation (64). Taken together, these studies suggest that MMPs may play a role in normal cardiovascular adaptation seen in normal pregnancies.

## 6. MATRIX METALLOPROTEINASES AND PREECLAMPSIA

As described above, preeclampsia is considered to be a two-stage disorder. In the first stage abnormal placentation leads to a release of circulating factors that ultimately produces the second stage of endothelial and vascular dysfunction. Many of the factors believed to cause preeclampsia induced vascular dysfunction have been shown *in vitro* to increase MMP activity. For instance, we have demonstrated that one of the postulated factors, vascular endothelial growth factor, increases MMP-2 release from endothelial cells in a concentration and time-dependent manner (65). Other studies have demonstrated that peroxynitrite, oxidized low density lipoprotein, and inflammatory cytokines can also activate MMPs *in vitro* (29, 30, 66). Thus, it is possible that multiple circulating factors promote MMP production and MMP release in this condition. As well, MMPs may promote the cleavage of syncytiotrophoblast microparticles that have been proposed to be a putative circulating factor that causes preeclampsia.

Supporting this theory are observations that MMP-2 activity is increased in the plasma of women with preeclampsia when compared to normotensive pregnant women (65). In a follow-up study to this initial observation, plasma samples were taken from pregnant women prior to the onset of preeclampsia (22 and 26 weeks) as well as at time of diagnosis with preeclampsia (67). Examining plasma from pregnant women prior to the clinical symptoms of preeclampsia is of particular interest since this plasma is capable of producing endothelial dysfunction when it is perfused into normal isolated blood vessels (68). Compared to plasma from gestational age matched normotensive pregnant women, MMP-2 activity was significantly increased in plasma even at 22 weeks (i.e. prior to the onset of clinical symptoms of preeclampsia).

At 26 weeks, although MMP-2 activity was equal between the two groups of women, the protein levels of TIMP-1 were significantly decreased in the plasma of women that subsequently developed preeclampsia. Thus, even at 26 weeks gestation an imbalance between MMPs and their endogenous inhibitors exists, suggesting an overall net increase in proteolytic activity. At the time of delivery/diagnosis of preeclampsia, MMP-2 activity was once again significantly elevated in the group of women with preeclampsia, confirming previous work (65). This study was of particular interest since the imbalance in MMPs preceded clinical disease, suggesting that MMPs may contribute to progression of preeclampsia from the first to second stage. Further studies could determine whether an imbalance in MMPs in the plasma contributes functionally to the clinical progression of this condition.

The role of circulating MMP-9 in preeclampsia still needs to be clarified. In one investigation circulating MMP-9 activity could not be quantified (65). In a second study, however, Myers et al (67) could quantify circulating MMP-9 activity, but no significant differences were seen between normotensive pregnant women and preeclamptic women. Interestingly, both MMP-2 and MMP-9 activities tended to increase over the duration of gestation. Although circulating levels of MMP-9 do not appear to be increased with preeclampsia, further studies will be needed to determine whether MMP-9 is affected in the vascular wall, where abundant activity of this proteinase has been detected (69).

As suggested above, increased proteolysis could be the result of increased MMP activity or decreased TIMP protein content. Myers et al (68) found that circulating TIMP-1 is decreased in preeclampsia, however Kolben et al (70) found no difference in this TIMP between women with preeclampsia and normotensive women. In the former study TIMP-2 was also measured but could not be quantified by immunoblot analysis. Thus, the status of circulating TIMPs in preeclampsia still remains to be determined.

A circulating imbalance in MMP activity may also have implications for cardiovascular disease postpartum. As mentioned previously, preeclampsia is associated with a significantly higher incidence of cardiovascular disease later in life (4). In studies investigating circulating MMPs in non-pregnant populations, perturbations in the MMP-TIMP system have been associated with hypertension and other cardiovascular complications (71, 72). Thus, it is possible preeclampsia induced changes in the MMP system could continue postpartum and eventually contribute to clinically significant cardiovascular disease. This hypothesis will need to be tested by future investigations.

An imbalance between MMPs and TIMPs has also been shown in preeclampsia through *in vitro* studies using cell culture models. Human umbilical vein endothelial cells from women with preeclampsia have increased release of MMP-2 compared to endothelial cells from normal pregnancies (73). In this investigation, the

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increase in MMP-2 activity was not accompanied by increases in TIMP-1 or -2 protein levels, which suggests an overall net increase in proteolytic activity. The fetal origin of the cells used in these experiments raises the possibility that modifications of the fetal cardiovascular system also occur during preeclampsia. These alterations in the fetus may contribute to a predisposition to increased blood pressure later in life (74).

In a separate set of experiments, preliminary results have demonstrated an increase in MMP-2 activity in endothelial cells incubated with plasma from women with preeclampsia, compared to cells incubated with plasma from normotensive pregnant women (75). TIMP-1, -2, and -3 protein levels were also decreased in cells incubated with plasma from women with preeclampsia. Taken together, these *in vitro* experiments suggest that the source of the circulating imbalance between MMPs and TIMPs in preeclampsia may be the dysfunctional endothelium.

As described above, studies have demonstrated both vasoconstrictive and vasodilator effects of MMPs. Thus, future investigations must uncover the functional role of MMPs in vascular regulation of women with preeclampsia. In a preliminary study, we determined the effect of MMP inhibition on mice mesenteric vessels incubated with plasma from non-pregnant, preeclamptic, or normotensive pregnant women (55). In these experiments, myogenic tone was enhanced in mice mesenteric arteries incubated with plasma from either preeclamptic or non-pregnant women. These two treatments also decreased endothelium-dependent relaxation to methacholine compared with vessels incubated with plasma from normotensive pregnant women. The addition of the broad-spectrum MMP inhibitor GM6001 further increased myogenic tone and further dampened vasorelaxation in preeclamptic plasma treated vessels, but not in the other two groups. These results suggest that MMP inhibition blocked the production of a vasodilator in these vessels. Many possible explanations exist for these results. MMP-2 may have cleaved big-ET to form ET[1-32] that acted on ET<sub>B</sub> receptors on the endothelium. Alternatively, the use of a broad spectrum MMP inhibitor may have blocked other MMPs that promote vasodilation. As well, the use of normal vessels with healthy endothelium may have produced results that may not reflect the situation seen *in vivo* with preeclampsia. Further studies in this area will unravel the exact mechanisms by which MMPs regulate vascular function in preeclampsia.

Aside from the MMP findings, another interesting aspect of this study was the similarity in vascular function between vessels treated with plasma from either non-pregnant or preeclamptic women. This similarity suggests that preeclampsia may be due, in part, to the lack of a necessary cardiovascular adaptation to pregnancy. Again, further studies will be needed to confirm this finding.

## 7. CONCLUSION AND PERSPECTIVES

Evidence suggesting that MMPs can contribute to the acute regulation of vascular tone continues to grow. In

the condition of preeclampsia it is likely that the role of MMPs as modulators of vascular tone will depend on the balance between the constrictor and dilatory properties of these proteinases. In the pro-inflammatory, pro-vasoconstrictive state of preeclampsia, the *in vivo* functional significance of the elevated levels of circulating MMP-2 remains to be determined. Unraveling the possible role of MMPs in preeclampsia could lead to a deeper understanding of the mechanisms involved in this condition. This greater understanding, in turn, could ultimately lead to alternative avenues of treatment.

## 8. ACKNOWLEDGEMENTS

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