Prolactin and vascular disease; a first cautious assessment

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Chapter 6

Prolactin fragmentation by trophoblastic matrix metalloproteinases as a possible contributor to peripartum cardiomyopathy and preeclampsia.

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ABSTRACT

Although peripartum cardiomyopathy (PPCM) is a rare disease, it has very serious consequences for both mother and child. No single cause has been held responsible for the pathogenesis. Recent studies have indicated that increased proteolytic cathepsin D activity in cardiomyocytes results in 16 kDa prolactin fragments with anti-angiogenic and apoptotic properties, which may contribute to the development of PPCM. In support of these findings, lowering full-length prolactin production by bromocriptine therapy has been reported to prevent impairment of cardiac function.

PPCM is associated with an increased co-existence of preeclampsia, however, a causal relationship has been disputed. We hypothesize that the pathophysiology of PPCM and preeclampsia share the same molecular pathway: increased activity of trophoblastic matrix metalloproteinases at the feto-maternal interface may aggravate proteolysis of full-length prolactin, and subsequently the formed 16 kDa prolactin fragments may contribute to deterioration of PPCM and preeclampsia. Therefore, we argue that it may be worthwhile to explore whether prolactin inhibition is not only beneficial for PPCM patients, but also for the much more prevalent preeclamptic women.

Introduction

Peripartum cardiomyopathy (PPCM) is a rare but life-threatening complication of pregnancy characterized by acute onset cardiac failure in previously healthy young women [1]. Hypertension is a known risk factor [1]. Preeclampsia is a pregnancy specific syndrome defined by hypertension and proteinuria and is in essence an endothelial disease [2]. It is a major contributor to perinatal and maternal morbidity and mortality. No causal therapy exists, except delivery of the placenta.

In this hypothesis-generating review, we will highlight some recent advances in understanding the pathophysiology of PPCM. Special attention is given to the cardio- and endotheliotoxic properties of the 16 kiloDalton (kDa) prolactin fragment, which is a cleavage product of full-length prolactin, and to the possible role of trophoblastic matrix metalloproteinases (MMPs) in prolactin fragmentation. These insights may offer new perspectives for future PPCM treatment policies. Fundamental and clinical investigation of rare diseases may bring to light pathophysiological mechanisms that might also play a role in other, more prevalent diseases. In addition, our attention has been drawn to the modulating role of 16 kDa prolactin fragments to endothelial dysfunction in preeclampsia.

PPCM and current treatment

The estimated PPCM prevalence is 1 per 3000 to 1 per 4000 pregnancies in the USA, but in other parts of the world, such as West Africa and Haiti, this disease is more prevalent [3]. It presents clinically in the last months of pregnancy and sometimes only in the first months after delivery as an acute dilating cardiomyopathy, leading to severe impairment of ventricular function and cardiac failure. Therefore, PPCM has serious maternal consequences in terms of risks of mortality and severe physical disability. The final diagnosis is made after exclusion of other causes that may lead to a similar clinical syndrome [1,4].

Overall mortality in women suffering from PPCM is close to 20%. Less than half of the patients who present with an ejection fraction of 25% or more will fully recover. An even worse prognosis with extended hospitalization and increased mortality is seen for those patients who have persistent reduced ventricular function more than 6 months after delivery.

The risk of PPCM recurrence in a subsequent pregnancy is high; in a retrospective cohort study of 44 women with PPCM in their medical history, almost 30% developed symptoms of heart failure in a subsequent pregnancy and almost 7% eventually died [5]. Even so, despite counseling women for the high chance of an adverse outcome, a next pregnancy remains under consideration by some of these patients [3,4]. Nowadays, only symptomatic treatment is available, consisting of supportive hemodynamic therapy with intravenous preload and afterload reducing agents or inotropic agents, diuretics and β-blockers. The combination of a dilated heart and the rapidly deteriorating cardiac performance often results in intraventricular development of thrombi, which can result in embolization and arterial occlusions of the lower extremities and parts of the brain. Prophylactic use of anticoagulant drugs, e.g. low molecular weight heparins (LMWH), has therefore been advocated [3,4].
Possible relation between PPCM and preeclampsia

Although PPCM is associated with increased preconceptional age, African origin, non-Caucasian ethnicity, poverty, multiparity, twin-pregnancy, prolonged use of tocolytic agents, and co-existence of preeclampsia, a number of studies have failed to associate these conditions with the a priori risk of PPCM, and a causal relationship has been disputed previously by several authors [1,3,4,6,7]. In fact, the etiology of PPCM is not well understood; viral antigens, an abnormal immune response to pregnancy, an abnormal response to hemodynamic stress, increased Gq-related myocyte apoptosis, cytokine-mediated inflammation, nutritional state, and a certain genetic predisposition may all play a role [1]. Although several molecular pathways have been proposed to explain PPCM, no overall concept exists, and we do not know who is at risk or how to prevent it. The same holds true more or less for preeclampsia [8]. In this review, we focus on recent studies that assign a key role to the generation of cardiovascular disease [9-12]. Whether prolactin and/or its fragments are indeed involved in the evolution of human atherosclerosis and its atherothrombotic complications is currently the focus of our research group, which contains the theme of cardiovascular endocrinology [13,14].

Prolactin and cardiovascular disease

The principal function of prolactin is to ensure lactation. Its physiological function outside pregnancy and the lactation period, if any, is unclear, especially in humans. Recent observations suggest that prolactin, either in excess or at physiological levels, might contribute to the pathogenesis of cardiovascular disease [9-12]. Whether prolactin and/or its fragments are indeed involved in the evolution of human atherosclerosis and its atherothrombotic complications is currently the focus of our research group, which contains the theme of cardiovascular endocrinology [13,14].

Regulation of prolactin production

Prolactin is synthesized and secreted by lactotrophic cells in the anterior pituitary gland in a circadian pulsatile rhythm with the highest levels in the early morning [15]. Pituitary secretion of prolactin is under inhibitory control of dopaminergic circuits that originate in the hypothalamus. Therefore, emotional and physical stress and certain centrally acting pharmacological agents (e.g. serotonin inhibitors), can raise prolactin levels in the circulation. Levels of prolactin vary in non-pregnant women between 4.0 and 25 μg/l [16]. In the course of pregnancy, prolactin attains serum peak levels that are close to 250 μg/l, implying a twenty-fold increase compared to reference values before pregnancy. During pregnancy, prolactin stimulates the maturation of the presecretory lobulo-alveolar system in the breasts, preparing the female body for lactation. After delivery, prolactin promotes galactopoiesis and the initiation of lactation [17]. Paradoxically, plasma prolactin concentration decreases markedly within several hours after delivery, even in women who are breastfeeding [18]. During lactation, extra pulsatile bursts of pituitary prolactin secretion occur in response to suckling. Kauppila and co-workers found that a woman with an isolated prolactin deficiency failed to lactate after two uncomplicated pregnancies, establishing the absolute necessity of pituitary prolactin production for lactation, but not for successful pregnancy outcome [19].

There are also multiple sites of extrapituitary prolactin release, such as lymphocytes, adipose tissue, myometrium, breast, prostate, and the decidualized endometrium in case of pregnancy [20]. The decidua even produces large amounts of prolactin that preferentially enters amniotic fluid, but little or none enters maternal blood. The main source of maternal prolactin serum levels is the anterior pituitary gland. At the extrapituitary sites, prolactin production and secretion is independently regulated by local factors [20]. Prolactin produced in the decidua may regulate immunological functions at the fetomaternal interface, placental angiogenesis, and amniotic fluid volume homeostasis [20]. This is a classical example of paracrine function between maternal and fetal tissues.

With regard to placental angiogenesis and endothelial cell proliferation, the intact, full-length, prolactin protein can enhance angiogenesis while the proteolytic 16 kDa fragment can inhibit angiogenesis [21]. In humans, several prolactin receptors have been identified. The long prolactin receptor isoform induces cell proliferation and cell survival in response to prolactin. The intermediate form is equipotent with the long form in mediating cell survival. When short prolactin receptors are co-expressed with the long form, they act as dominant negatives [20]. Therefore, the role of prolactin in regulating placental vascularisation may vary at different periods of gestation based on the type of receptors expressed and the relative form of prolactin present.

Prolactin fragments and PPCM

Upon secretion, either by the pituitary gland or at extrapituitary sites, prolactin has a molecular weight of 23 kDa. This full-length pituitary stimulates angiogenesis. However, 16 kDa prolactin fragments have the exact opposite effect [22]. Clapp and co-workers defined vaso-inhibins as a family of peptides, including the 16 kDa fragment of prolactin, with potent anti-angiogenic, vasoconstrictive and apoptotic properties [23-25]. A dysbalance between 23 kDa and 16 kDa prolactin, in favor of the latter, can promote apoptosis and inhibit angiogenesis [22]. Hilfiker-Kleiner et al. hypothesized that this dysbalance may play a detrimental role in PPCM [10,26]. To test this hypothesis, Hilfiker-Kleiner et al. used an experimental model with mice that suffer from increased oxidative stress in the heart as a result of a lack of STAT3 in cardiomyocytes [10]. STAT3 is involved in protection of the heart from oxidative stress by upregulation of antioxidative enzymes, such as reactive oxygen species (ROS) scavenging enzyme and manganese superoxide dismutase (MnSOD) [27]. Indeed, these STAT3 null mice, when pregnant, developed a form of heart failure, which closely resembles PPCM. The oxidative stress results in increased ventricular levels of cathepsin D, a cardiac enzyme capable of cleaving 23 kDa prolactin into its 16 kDa fragments. As expected, higher levels 16 kDa prolactin were demonstrated in the left ventricle of STAT3 null mice, as compared to wild-type mice. In order to lower circulating prolactin levels and its fragments, pregnant STAT3 null mice were subsequently treated with bromocriptine, a dopamine receptor agonist that is used in the treatment of hyperprolactinemia. Indeed, upon treatment, deterioration of cardiac performance was prevented, with a marked decrease in postpartum mortality.

In additional experiments an adenoviral vector expressing human 16 kDa prolactin was injected in the left ventricle in pregnant wild-type mice. This resulted in left ventricular dilatation and decreased...
Prolactin fragmentation as a possible contributor to PPCM and PE

Cardiac function, and immunohistochemistry showed a decrease in left ventricular capillary density [10]. Preliminary data from human studies by the same research group further support their hypothesis that 16 kDa prolactin fragments might play a role in the development of PPCM. They found increased levels of oxidized low-density lipoprotein, an accepted biomarker for oxidative stress in women with PPCM [10]. In line with their observations in mice, elevated cathepsin D activity was also found in these patients, and 16 kDa prolactin fragments were generated as well. In healthy pregnant women on the other hand, predominantly full-length 23 kDa prolactin was present in the circulation, whereas 16 kDa prolactin fragments were below the detection limit [10].

**Trophoblastic MMPs and proteolytic activity**

The central issue in this new pathophysiological concept of PPCM is to identify the source of proteolytic activity leading to prolactin fragmentation. Until now, investigators have focused on myocardial cathepsin D. However, we think that the source of proteolytic enzymes does not necessarily have to be the heart itself. Instead, we argue that MMPs of trophoblastic origin deserve special attention. MMPs are a family of proteolytic enzymes that are involved in the remodeling and physiological homeostasis of the extracellular matrix [28]. Indeed, baseline serum MMP-2 levels have been shown to be significantly higher in 43 PPCM patients compared with 20 pregnant controls [29]. This suggests a role of MMPs in the pathophysiology of PPCM, possibly, so we hypothesize, through prolactin fragmentation.

In normal pregnancy, cytotrophoblastic cells produce MMPs which are involved in the physiological trophoblastic invasion of the maternal-placental vascular bed in early pregnancy [30,31]. We hypothesize that their action may be direct and/or indirect, since MMPs are also capable of cleaving full-length prolactin into 16 kDa prolactin fragments: MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 produced and secreted by chondrocytes, have been shown to be able to cleave human prolactin into biologically functional 16 kDa fragments [32]. We propose that trophoblastic MMP activity and decidual prolactin production may lead to local prolactin proteolysis, resulting in 16 kDa fragments at the feto-maternal interface, thereby modulating trophoblastic ingrowth in the spiral arteries (Figure). This physiological process may become pathological when the production of prolactin fragments is elevated, since these endotheliotoxic fragments might enter the maternal circulation with detrimental effects for the maternal heart.

**MMP activity as a modulator of both preeclampsia and PPCM**

The same molecular pathway could play a role in preeclampsia, explaining the epidemiological association between preeclampsia and PPCM. The idea that prolactin is involved in the etiology of preeclampsia is far from new: Already in 1975 it was suggested that either elevated prolactin levels, or an increased responsiveness to normal prolactin levels, could play a key role in the pathogenesis of preeclampsia, since it was known that prolactin could elevate arterial pressure [33]. The idea that predominant decidual production of 16 kDa prolactin contributes to the etiology of preeclampsia is not novel either: Parra et al. argued that immune maladaptation to pregnancy may contribute to predominant decidual production of 16 kDa prolactin [34].

Preeclampsia often arises from pre-existing vascular compromise [35,36]. The established root-process in the development of preeclampsia is inadequate trophoblast invasion in the spiral arteries in early pregnancy [37]. All subsequent clinical symptoms characterizing preeclampsia, such as hypertension, edema, proteinuria, liver and kidney damage, eclamptic seizures, and coagulopathy, ultimately result from generalized endothelial lesions [35]. The impairment of trophoblast invasion may involve MMPs. Despite the discrepancies across studies discussing MMP levels and activity in association with preeclampsia, a growing body of evidence demonstrates that MMPs do play a role in mediating vasodilatation and reducing myogenic tone in pre-eclamptic women [38]. MMP activity is predominantly modulated by specific tissue inhibitors of MMPs (TIMPs). The balance between MMPs and TIMPs is likely to play an important role in the remodeling of uterine arteries in pregnancy, and it may contribute to the maintenance of vasodilatation in later pregnancy. Recently it was shown that MMP-2 and TIMP-1 values are significantly higher in preeclampsia [39]. Another study confirms that plasma MMP-2 levels are significantly elevated in preeclamptic women elevated at 22 weeks of pregnancy ($p=0.02$) and at diagnosis ($p=0.003$). However, in this study, there were no significant differences in the expression of MMP-9 between the groups at the gestational time points analyzed [40]. In contrast, Poon et al. did find increased levels of MMP-9 in pregnancies developing preeclampsia [41].
might become part of future preventive strategies. The underlying idea is that lowering full-length
for PPCM patients. And, given the high recurrence rate of PPCM, dopamine receptor agonist therapy
still in an experimental setting, lowering of prolactin levels per se might contribute to better outcomes
cardio-endotheliotoxic effects of 16 kDa prolactin fragments. In line with these observations, although
these new insights might provide clinicians with new treatment options for PPCM and possibly even
possible contributor to PPCM and PE

Although the exact role of MMPs in the pathophysiology of preeclampsia is still unclear, the majority
of recent literature suggests that enhanced MMP activity may be related to preeclampsia. Therefore,
we propose that MMPs may be involved in the pathophysiology of preeclampsia, either directly, or
indirectly, via proteolysis of full-length prolactin. We think that increased MMP levels may lead to a
dysbalance between full-length prolactin and 16 kDa fragments, which could explain both inadequate
early placentation and the later occurring endothelial dysfunction that is central to the pathophysiology
of preeclampsia. The finding of elevated levels of 16 kDa prolactin fragments in the circulation, urine,
and amniotic fluid of preeclamptic women fits in this model [42]. Thus, elevated MMP activity might play
a similar role in the development of preeclampsia as in PPCM; consequently, the reported association
between the two disease entities does not necessarily need to be causal. Instead, we hypothesize that
both diseases are aggravated by prolactin proteolysis through trophoblastic MMP activity. We suggest
that, in women with PPCM, besides cathepsin D secretion by the maternal heart, the impaired placental
circulation might serve as an additional source of proteolytic activity (i.e. MMP secretion), leading
to generation of 16 kDa prolactin fragments, subsequently deteriorating cardiac condition in PPCM
patients (Figure). In line with this, placental 16 kDa prolactin fragments, generated by trophoblastic
MMPs, could aggravate endotheliopathy in preeclamptic women (Figure). Variation at the level of the
genome may lead to inter-individual variation in MMP protein activity, explaining why only a small
minority of pregnant women, even if complicated by preeclampsia, develop PPCM [43]. We postulate
that this genomic variation could also play a role in preeclampsia, however, the MMP activity needed
to modulate PPCM may not be as high as for PPCM, explaining why preeclampsia may be a more
frequently occurring disease.

In conclusion, we propose MMP-induced prolactin fragmentation as a modulator in the development
of both PPCM and preeclampsia. To actually test this hypothesis, it would be necessary to isolate
MMPs from trophoblasts of women suffering from PPCM and/or preeclampsia. Subsequently, we
would need to evaluate whether MMPs isolated from these trophoblasts are capable of cleaving
human full-length prolactin into 16 kDa prolactin. At the same time, expression of prolactin in these
placentas should be confirmed. It has already been shown that the serum, urine, and amniotic fluid
obtained from women with severe preeclampsia contain significantly higher levels of 16 kDa prolactin
compared to the biological fluids of normal pregnant women [42]. Furthermore, serum levels of 16
kDa prolactin are elevated in PPCM patients [10]. We recognize that isolation of trophoblastic MMPs
would be an extremely difficult task.

Possible clinical implications and future research
These new insights might provide clinicians with new treatment options for PPCM and possibly even
for preeclampsia. As described above, preliminary observations in both mice and humans suggest
cardio-endotheliotoxic effects of 16 kDa prolactin fragments. In line with these observations, although
still in an experimental setting, lowering of prolactin levels per se might contribute to better outcomes
for PPCM patients. And, given the high recurrence rate of PPCM, dopamine receptor agonist therapy
might become part of future preventive strategies. The underlying idea is that lowering full-length
prolactin levels will also reduce proteolysis into prolactin fragments. A few case reports described
dramatic improvement in cardiac function of PPCM patient after treatment with dopamine agonists
[26,44-46]. A small clinical intervention study (n=12) provided additional support for the prolactin-PPCM
hypothesis; in women with a PPCM history prophylactic treatment with bromocriptine until 3 months
postpartum preserved normal heart function in six patients, whereas left ventricular ejection fraction
in the six control patients, who only received standard supportive therapy, did deteriorate and three of
these women died [10]. In the intervention group (n=6), prolactin levels returned to non-pregnant levels
within 14 days of bromocriptine treatment and left ventricle function was preserved. All six patients
survived during four months follow-up. These promising results of animal and human experiments,
all by one group, will have to be reproduced by other, independent researchers before drawing any
definitive clinical conclusions. 16 kDa prolactin fragments have been shown to be increased in the
serum, urine, and amniotic fluid of preeclamptic women, suggesting that these fragments contribute
to the endothelial cell dysfunction that characterizes this disease [42]. Whether preeclamptic women
will benefit from prolactin lowering therapy has not been investigated yet.

It should be emphasized, that the safety-profile and effectiveness of dopamine receptor agonists
in human PPCM treatment has not yet been established sufficiently. In the standard treatment of
hyperprolactinemia with bromocriptine, several rare but serious adverse events have been reported
including psychosis, myocardial infarction, possibly due to coronary vasoconstriction, strokes and
maternal hypertension [1]. For this reason, the US Food and Drug Administration does not allow
bromocriptine anymore for the suppression of lactation in the postpartum period. The alternative
cabergolin is similarly disadvised; cabergolin is not the first choice in most international guidelines
for the treatment of prolactinomas in pregnancy and a high dose of cabergolin for suppression of
lactation has been associated with cardiac valvular disease and cardiac outflow disturbances [47].
However, these rare complications do not necessarily preclude the potential use of dopamine receptor
agonists for life-threatening diseases such as PPCM and/or severe early onset preeclampsia, provided,
of course, that the benefits are clear.

In conclusion, it may be difficult to really test the hypothesis that trophoblastic MMPs and prolactin
fragments indeed play a role in PPCM and preeclampsia. Nonetheless, the hypothesis may open a new
field of research, both fundamental and clinical, and to our opinion it seems worthwhile to explore
whether PPCM patients or severely ill preeclamptic women will benefit from prolactin lowering therapy.

Disclosures
None.

Conflicts of interest statement
None declared.
Activation of signal transducer and activator of transcription 3 protects Peripartum cardiomyopathy–a new treatment option by inhibition of


