Hemopexin activity and extracellular ATP in the pathogenesis of preeclampsia

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General introduction
During pregnancy, many essential physiological adaptations occur in the mother to support growth of the developing foetus. Cardiac output and blood volume e.g. increase by approximately 50%, and there is a slight decrease in blood pressure [1-3]. To facilitate expansion of the vascular bed, peripheral vascular resistance decreases, partly due to decreased vascular responsiveness to angiotensin II [4]. In addition, the maternal immune system has to tolerate the presence of the semi-allogeneic foetus, and therefore systemic and local alterations in the immune system occur during pregnancy [5]. In general, the innate immune system becomes activated, while the adaptive immune system shifts towards a type 2 immune response, with increased numbers of regulatory T cells [5]. These maternal adaptations to pregnancy are necessary for the development of a whole new organ, i.e. the placenta, and the growing foetus. The importance of the development of these changes during pregnancy is supported by the association between aberrances in these adaptations and pregnancy complications such as pre-term labour [6] and preeclampsia [4,7].

**Preeclampsia**

Preeclampsia is one of the major pregnancy complications in the Western world. It occurs in 3-5% of the pregnancies [8] and is mainly characterized by hypertension, proteinuria and oedema in the second half of pregnancy [7]. So far, the only cure for preeclampsia is delivery of the foetus and the placenta. Even though after delivery the symptoms vanish, it has become obvious in the recent years that women with preeclampsia are more prone to develop renal and cardiovascular diseases later in life [9,10].

**Pathogenesis of preeclampsia**

The current knowledge on preeclampsia is increasing, however, its pathogenesis remains elusive. It has been generally accepted that preeclampsia may be a two-stage disease. Initially, in the first trimester poor placentation occurs, which is clinically symptomless [11]. Poor placentation in preeclampsia is characterized by defective trophoblast invasion and spiral artery remodelling in the first trimester of pregnancy [12]. During normal placentation, foetal trophoblast cells invade into the maternal spiral arteries and replace the endothelial lining. Smooth muscle cells are degraded and the arteries are remodelled into wide rigid vessels that are nonresponsive to vasoactive hormones, like angiotensin II [12]. Poor placentation in preeclampsia is thought to disturb normal blood flow in the placenta and increases the velocity and spurting of the blood flow, leading to oxidative stress and hypoxia [13]. This leads to the second stage of the disease [11,14], in which the damaged and stressed placenta is believed to release many factors into the maternal blood, such as anti-angiogenic factors (sFlt-1 and sEng), syncytiotrophoblast microparticles (STBMs), cytokines and DAMPs (such as extracellular ATP) [15-17]. These factors are thought to induce endothelial damage and stimulate activation of endothelial cells and immune cells, leading to maternal systemic endothelial dysfunction and systemic inflammation [11]. Systemic endothelial dysfunction...
and inflammation is subsequently thought to initiate the development of the main symptoms of the disease, i.e. proteinuria, hypertension and oedema in the second half of pregnancy [11].

**Hemopexin**

Hemopexin (Hx) is a free heme scavenger, able to bind heme with the highest affinity, and is mainly produced in the liver [18]. It is released in the circulation, where it mainly functions as an anti-oxidant to bind heme, protecting cells from heme mediated toxicity [18,19]. Hx is also an acute phase protein, being produced in inflammatory conditions [18]. Hx is not only a heme scavenging protein but also possesses serine protease activity [20]. This protease activity can be inhibited by extracellular nucleotides, such as ATP [21]. Moreover, proteolytic active Hx can damage the endothelium. In vitro, active Hx downregulated glomerular ecto-apyrase, and intra-renal infusion of Hx in rats induces proteinuria [22].

**Hx activity during pregnancy and in preeclampsia**

Hx activity was shown to be increased in patients with minimal change disease in relapse [23], and was thought to contribute to symptoms like proteinuria and oedema in these patients. As similar symptoms are observed in women with preeclampsia, and also healthy pregnancy is associated with a tendency to renal protein leakage [24] and mild oedema [25], we became interested in alterations in Hx activity during healthy pregnancy and in women with preeclampsia.

It was found that Hx activity was increased in pregnant women as compared to nonpregnant women [15]. The Hx activity started to rise around week 10-15 of pregnancy, and remained high until the end of pregnancy [26]. At the same time point during pregnancy, around week 10-15, the vascular system of pregnant women becomes less responsive to angiotensin II [4]. Since active Hx was shown to be able to shed the receptor for angiotensin II, the AT-1 receptor (AT-1R) from endothelial cells and monocytes in vitro, Hx activity may contribute to the decreased sensitivity to angiotensin II by decreasing AT-1R availability during pregnancy [26,27].

In contrast, in women with preeclampsia, the plasma Hx activity is decreased to values similar to the nonpregnant state [15]. The decreased Hx activity may be due to increased levels of extracellular ATP, the inhibitor of Hx activity, which is increased in plasma from women with preeclampsia compared with healthy pregnant women [15]. It was hypothesized that deceased Hx activity resulted in decreased shedding of the AT-1R and therefore contributed to the increased responsiveness to angiotensin II in women with preeclampsia.

**Extracellular ATP in preeclampsia**

Although ATP is mainly known as an intracellular source of energy, in the recent decades it has become clear that ATP can also be released from cells into the extracellular environment.
In the extracellular space ATP serves as a danger signal or danger associated molecular pattern (DAMP) [28], since ATP release mainly occurs from stressed or necrotic cells [29]. Extracellular ATP binds to purinergic P2 receptors, which are expressed on many cell types and tissues such as immune and endothelial cells [28,30] and in the placenta [31]. Once in the extracellular space, ATP is immediately hydrolysed by membrane bound ATP hydrolysing enzymes on the plasma membrane, such as ectonucleotidases (CD39 and CD73) and alkaline phosphatase (AP), into adenosine. Adenosine has opposing functions compared with ATP and therefore the balance between these two molecules determines their net effects (see Chapter 2, Figure 1). During pregnancy, the ATP/adenosine balance changes due to increased adenosine levels [32], while in preeclampsia this balance shifts to the ATP induced effects, due to increased ATP levels [15] as compared with adenosine [33]. Therefore, high ATP levels during pregnancy, as observed in women with preeclampsia, could be toxic during pregnancy due to its pro-inflammatory effects.

**Rat model for preeclampsia**

To study the potential pathophysiological role of ATP in pregnancy, we used a rat model. Rats may be best suitable for studies related to pregnancy [34], since, human and rat placentation are quite similar. Placentation in both humans and rats is of the hemochorial type, in which the foetal trophoblast cells are in direct contact with the maternal blood and uterine tissues. Moreover, deep invasion of trophoblast cells that is observed in human pregnancy also occurs in rats in the mesometrial triangle, the equivalent of the human placental bed [35,36]. Like in the human situation, spiral artery remodelling also occurs in rats [34], and this is supported by leukocytes (NK cells and macrophages) present in the mesometrial triangle [37]. However, the timing of trophoblast invasion into the uterine wall is earlier in gestation in humans (before 20 weeks) [38] compared with rats (in the last week) [35].

As rat pregnancy may be the best suitable alternative for human pregnancy-related studies, we previously developed an animal model for preeclampsia in which infusion of ATP on day 14 of pregnancy induced preeclampsia-like symptoms [39]. These included proteinuria, decreased foetal weight and placental ischemia at the end of pregnancy, and were exclusively observed in pregnant animals [39]. In addition, these rats also showed decreased plasma Hx activity. However, the exact mechanisms of how ATP and Hx activity induced their effects in pregnancy and preeclampsia remained to be investigated.

**Aims of the thesis**

Based on the above mentioned animal model, which has shown that ATP is toxic for pregnant rats and induced preeclampsia-like signs [39], the first aim of this thesis was to investigate how ATP induces the signs of preeclampsia in the pregnant rat. The second aim of this thesis was to further investigate the role of Hx activity in pregnancy and preeclampsia.
Part I: The effect of ATP on pregnancy

As ATP is a pro-inflammatory stimulus we postulated that it may induce preeclampsia-like effects via activation the immune system. Therefore our main focus was on the effect of ATP on the immune response during pregnancy, which we assessed in experimental studies in rats with the above described ATP model.

- In Chapter 2, a review provides more insight into how high ATP levels may potentially contribute to the pathogenesis of preeclampsia via effects on blood pressure and the immune response.
- In Chapter 3 we focused on the effects of ATP on peripheral blood monocytes, since monocytes are important cells of the circulating innate immune system and are increased and activated in preeclampsia [40,41]. We investigated the effect of ATP infusion on monocyte subsets and compared the results to human preeclampsia.
- In Chapter 4 we studied whether other immune cells, granulocytes and T lymphocytes, were also affected by ATP infusion in pregnant rats. In addition, as the kidney is one of the organs affected by ATP in rats [39], and in women with preeclampsia [42], in this chapter we also assessed whether ATP induced an inflammatory response in the kidneys of these rats on day 15, 17 and 20 of pregnancy.
- Finally, in Chapter 5 we studied the effect of ATP on placentation in rats. As ATP infusion decreased foetal weight, we investigated if ATP affects trophoblast invasion and spiral artery remodelling on day 15, 17 and 20 of pregnancy, and whether this was associated with alterations in the inflammatory response in the rat mesometrial triangle.

Part II: The effect of Hx activity on pregnancy

In the second part of this thesis we tested the hypothesis that Hx activity sheds the AT-1R in vivo and we studied whether reactivation of Hx activity and decreasing ATP levels by the ATP hydrolysing enzyme alkaline phosphatase (AP) could increase Hx activity and decrease preeclampsia-like symptoms in pregnant rats.

- In Chapter 6, more insight into the physiological role of Hx activity during pregnancy and how active Hx may contribute to decreased Angiotensin II sensitivity in pregnancy was reviewed.
- In Chapter 7, we evaluated whether active Hx could shed the AT-1R in vivo. Therefore, we investigated in vivo the relation between Hx activity, ATP levels, tissue AT-1R expression and plasma AT-1R levels in healthy pregnant and non-pregnant women, as well as in early- and late-onset preeclamptic women.
- In the final chapter of this thesis, Chapter 8, we aimed to investigate whether reactivation of Hx activity and decreasing ATP levels would decrease preeclampsia-like symptoms in pregnant rats. To test this we treated ATP infused pregnant rats with the ATP-hydrolysing enzyme AP and tested whether we were able to prevent proteinuria, glomerular inflammation and disturbed trophoblast invasion in these animals.
References


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Danger signals from ATP and adenosine in pregnancy and preeclampsia

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