Vascular dysfunction in preeclampsia
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Chapter 10

Vascular remodeling in pregnancy and preeclampsia


Submitted.
Abstract

Objective: To determine whether preeclampsia is associated with maladaptive vascular remodeling.

Methods: Resistance arteries were isolated from subcutaneous fat biopsies, obtained during cesarean delivery or abdominal surgery from preeclamptic (n=14), normal pregnant (n=21) and nonpregnant women (n=8), and mounted in a pressure myograph. Plasma samples were obtained from preeclamptic women at hospital admission and from age- and gestational age-matched normal pregnant and nonpregnant women (n=24 each).

Results: Passive mechanical properties and wall thickness to lumen ratios of subcutaneous arteries were similar in the groups. Matrix metalloproteinases (MMP) 2 and 9 were determined by zymography. Active MMP-9 was increased in normal pregnancy compared with nonpregnant women (260 ± 79 vs 192 ± 65 intensity units, P=.008) and further increased in preeclampsia (334 ± 84 intensity units, P=.03).

Conclusion: We found no direct evidence for vascular remodeling in isolated subcutaneous arteries in pregnancy and preeclampsia. However, the increased plasma activity of MMP-9 in these conditions could reflect ongoing matrix degradation and thus remodeling.
Resist ance artery remodeling

Introduction

Normal pregnancy is associated with extensive anatomical and functional changes of the cardiovascular system to accommodate the demands of pregnancy. In preeclampsia this adaptation is inadequate. This condition is mainly characterized by a generalized vascular dysfunction, resulting in an increased peripheral resistance and hypertension. Whether the increased peripheral resistance results from increased microvascular tone, a quantity that may change in seconds, or from remodeling of the resistance vasculature, a process that takes days [299], is unclear. The rapid recovery of preeclampsia, within days after delivery, is consistent with both processes. However, the possibility that pregnancy and preeclampsia are associated with vascular growth or remodeling of the resistance vasculature deserves serious consideration since it has consequences for the possible role of growth factors and for antihypertensive or anti-remodeling therapy [300].

No straightforward approach is available to assess the structure of the resistance vasculature longitudinally. Moreover, on cross-sectional studies it is difficult to make comparisons between groups due to biases introduced by selection of vessels from irregularly branching microvascular networks of individual patients [301]. Still, there is some evidence that suggests the involvement of remodeling in preeclampsia. In isolated omental arteries from women with preeclampsia, the ratio of wall thickness to lumen diameter is reported to be increased, compared with that in vessels from normal pregnant women and nonpregnant women [150].

In previous studies on animal vessels in organoid culture we observed that inward remodeling is associated with a change in the passive pressure-diameter curve [151]. In particular, pressurization caused less distention of the remodeled vessels as compared to controls [151,299]. Thus, a comparison of the distensibility of resistance vessels from preeclamptic, normal pregnant and nonpregnant women could provide information on ongoing remodeling even though the location of the isolated vessel segments within their networks may be different.

In this study we tested whether microvascular inward remodeling occurs in preeclampsia. In particular, we determined passive properties of isolated subcutaneous arteries from the resistance vasculature of preeclamptic, normal pregnant and nonpregnant women women. In addition, we considered that vascular remodeling requires adaptation of the extracellular matrix, notably the collagen backbone in the vascular wall. Accordingly, concentrations and activation status of two of the involved MMP’s, MMP-2 and MMP-9, were determined in the plasma’s of women with preeclampsia, normal pregnant and nonpregnant women.

Methods

Properties of isolated subcutaneous arteries

The medical ethical committee of the Academic Medical Center approved the study. After obtaining informed consent, subcutaneous fat biopsies were taken during cesarean delivery from women with preeclampsia and normal pregnant women and at elective abdominal surgery from
nonpregnant women. The samples were immediately placed in ice-cold PSS. From the subcutaneous fat resistance arteries of about 200 μm in diameter were dissected and mounted on two glass cannulas in a pressure myograph. The chamber was filled with PSS, which was continuously refreshed. Some of the included arteries were also used for the experiments described in chapter 10. After a calibration period of approximately 30 minutes, arteries were pressurized to 100 mmHg in calcium-free PSS containing EGTA 10 mmol/L and ionomycin 2 μmol/L. The passive pressure-diameter relation was then determined at pressures of 5, 10, 20, 30, 60, 80 and 100 mmHg. The diameter at all intraluminal pressures was calculated as a percentage of the passive diameter at 100 mmHg. The lumen diameter and wall thickness, as imaged by a CCD camera attached to the microscope, were measured at each pressure and analyzed with the use of own-built software. The wall thickness to lumen ratio was calculated.

**Measurement of MMP-2 and MMP-9 in plasma**

After obtaining informed consent, plasma samples were obtained from separate groups of women, with preeclampsia or a normal pregnancy, matched for age (± 5 yr) and gestational age (± 2 wk) and nonpregnant women, matched with the women with preeclampsia and the normal pregnant women for age (± 5 yr). Blood samples were taken from the antecubital vein without tourniquet through a butterfly needle with a vacutainer system into a 4.5 mL tube containing 0.105 mol/L citrate (Becton Dickinson; San Jose, USA). Cells were removed by centrifugation for 20 minutes at 1550g at room temperature. Plasma samples were divided in aliquots of 250 μL, snap frozen in liquid nitrogen and stored at -80°C until tests were performed. 1 μL of each plasma sample was dissolved in 10 μL sample buffer (sucrose 20%, Tris-HCl 125 mmol/L, SDS 4%, EDTA 10 mmol/L and bromophenol blue 0.05%) and loaded on a standard polyacrylamide gel, containing 10% SDS and 0.2% gelatin. Samples of matched subjects were loaded on the same gel. The samples were run at 50mA through the gel. The gel was then rinsed in a Tris-HCl buffer (50mM, pH 7.5) containing 5mM CaCl₂ and 2.5% Triton for 15 min and then incubated (overnight at 37°C) in a Tris-HCl buffer (50mM, pH 7.5) containing 5mM CaCl₂ with 1% triton. The overnight incubation was followed by 2 short washes in aquadest, staining the gel with Coomassie blue 0.1% for 2 hrs. at 60°C and destaining of the gel in a 7% acetic acid and 4% methanol solution for 1 hr. at 60°C. This results in clear white bands against a blue background. The white bands for MMP-2 and MMP-9 (both active and inactive) were identified on basis of their molecular weights (72 and 92 kDa, respectively), which were determined by comparison with protein molecular weight standards (Bio-Rad high range: myosin 213 kDa, β-galactosidase 120 kDa, BSA 76 kDa, ovalbumin 47 kDa). The white bands were quantified using a densitometric method on a GelDoc 2000 system (Bio-Rad) using the Quantity One 4.2.0 software (Bio-Rad). All bands were normalized for background density. The average intensity of each band was determined and multiplied with the broadness of the band to determine the MMP levels.
Statistical analysis

Differences in the groups were determined with ANOVA with a Bonferroni post hoc test. Differences in patient characteristics, passive mechanical properties of arteries and in wall thickness to lumen ratio between groups were determined with ANOVA with a Bonferroni post hoc test. Since matched subjects were loaded on the same gels, in order to exclude effects of inter-gel variability, differences in MMP levels between preeclamptic and normal pregnant women, and between nonpregnant and normal pregnant women were determined with paired student t tests with a Bonferroni correction. Differences were considered statistically significant with P < .05. All data are presented as means ± SEM.

Results

Subjects

The characteristics of the women from whom subcutaneous fat biopsies or plasma were obtained are presented in table I. Women with preeclampsia had higher systolic and diastolic blood pressures and significant proteinuria. Ten women also fulfilled the criteria for the (H)ELLP syndrome, four in the subcutaneous fat biopsy group and six in the plasma group. The preeclamptic women delivered at earlier gestational ages of children with lower birthweights. In three women with preeclampsia, who contributed plasma, perinatal death occurred.

Properties of isolated subcutaneous arteries

The passive diameter of the isolated arteries at 100 mmHg was similar in the three groups (182 ± 18 μm in the preeclamptic vessels, 173 ± 11 μm in the normal pregnant vessels and 163 ± 17 μm in the nonpregnant vessels). Being part of heterogeneous microvascular networks these passive diameters are not directly comparable. However, their resemblance in diameter indicates that similar vessels were isolated from the three groups. There were no differences between the three groups in passive mechanical properties of the isolated subcutaneous arteries, as indicated by the pressure-diameter relationships (figure 1). Wall thickness to lumen ratios were also similar at all pressures. Wall thickness to lumen ratios are presented at 60 mmHg in figure 2.

Plasma MMP-2 and MMP-9 levels

Figure 3 shows an example of a zymographic analysis of MMP levels in the plasma of a matched set of women, one with preeclampsia, a normal pregnant woman and a nonpregnant woman. Inactive MMP-2 was present in the plasma of all women and concentrations were similar in all groups (figure 4). In none of the women active MMP-2 was detected. Inactive MMP-9 tended to be increased in normal pregnancy compared with nonpregnant women and women with preeclampsia (702 ± 88 vs 558 ± 58 and 574 ± 57 intensity units, P = .07 and P = .10, respectively). Active MMP-9 was increased in normal pregnancy compared with nonpregnant...
women (260 ± 79 vs 192 ± 65 intensity units, \( P = .008 \)) and further increased in preeclampsia (334 ± 84 intensity units, \( P = .03 \)).

**Table I. Patient characteristics.**

<table>
<thead>
<tr>
<th>Isolated arteries</th>
<th>Preeclampsia</th>
<th>Normal pregnant</th>
<th>Nonpregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>n =</td>
<td>14</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30.0 ± 1.0</td>
<td>33.4 ± 1.0</td>
<td>33.8 ± 1.6</td>
</tr>
<tr>
<td>Gestational Age (wk)</td>
<td>30.1 ± 2.2*</td>
<td>39.1 ± 0.6</td>
<td>-</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>1473 ± 192*</td>
<td>3332 ± 187</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>111.8 ± 2.0*</td>
<td>73.1 ± 2.0</td>
<td>74.6 ± 2.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>173.5 ± 6.4*</td>
<td>112.4 ± 3.0</td>
<td>121.6 ± 6.6</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>5.4 ± 1.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**MMP measurement**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Preeclampsia</th>
<th>Normal pregnant</th>
<th>Nonpregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>n =</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31.1 ± 1.0</td>
<td>31.0 ± 1.0</td>
<td>31.0 ± 1.0</td>
</tr>
<tr>
<td>Gestational Age (wk)</td>
<td>30.4 ± 0.8</td>
<td>30.3 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>102.3 ± 1.9*</td>
<td>64.5 ± 2.3</td>
<td>71.5 ± 1.6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>157.3 ± 3.3*</td>
<td>108.6 ± 4.0</td>
<td>111.3 ± 2.4</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>4.6 ± 0.8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Delivery**

| Gestational Age (wk)                     | 32.0 ± 0.7*  | 39.6 ± 0.4      | -           |
| Birthweight (g)                          | 1368 ± 133*  | 3592 ± 116      | -           |

*Demographic data of the women with preeclampsia, normal pregnant and nonpregnant included in the study, who contributed either a subcutaneous fat biopsy or plasma.

*ANOVA \( P < .001 \)
Figure 1. Passive mechanical properties of isolated subcutaneous arteries from women with preeclampsia (black circles, n=14), normal pregnancy (open circles, n=21) and nonpregnant women (grey triangles, n=8) at increasing intraluminal pressures.

Figure 2. Wall thickness to lumen ratio at 60 mmHg in isolated subcutaneous fat arteries of women with preeclampsia (black box, n=13), normal pregnancy (white box, n=21) and nonpregnant women (grey box, n=7).
Figure 3. Zymographic analysis of plasma samples from a matched set of women, a woman with preeclampsia (PE), a normal pregnant (PR) and a nonpregnant (NP) woman. The predominant band, which runs with a MW of 72 kDa (C) represents inactive MMP-2. The smaller band, with a MW of 92 kDa (A) represents inactive MMP-9. In the row with the plasma of the woman with preeclampsia an extra band can be seen below the 92 kDa band (B), which represents active MMP-9.

Figure 4. Expression of inactive MMP-2, inactive and active MMP-9 in the plasma of women with preeclampsia (black boxes, n=24), normal pregnancy (white boxes, n=24) and nonpregnant women (grey boxes, n=24).

* Paired t test P < .01 vs nonpregnant women
** Paired t test P < .05 vs normal pregnant women
Comments

Microvascular distensibility at full dilatation and wall thickness to lumen ratios provided no indications for the presence of established vascular inward remodeling in isolated subcutaneous arteries in pregnancy and preeclampsia. However, the level of circulating active MMP-9 was increased in normal pregnancy and further increased in preeclampsia. Since MMP-9 is involved in alterations in extracellular matrix composition. This finding indicates the possibility of ongoing systemic vascular remodeling in normal pregnancy and in preeclampsia. There are several important considerations regarding the used techniques that could explain the discrepancy between the information obtained from isolated arteries and from circulating MMP's.

First, it should be emphasized that we have investigated remodeling in isolated subcutaneous arteries and it may be argued whether these arteries are representative for the whole resistance circulation. However, it is generally assumed that alterations in the vasculature in pregnancy and preeclampsia are systemic, and subcutaneous vessels have been used as a representative source on many occasions. Therefore, one would expect that when remodeling occurs in the vasculature it should also be found in the subcutaneous vascular bed. Nevertheless, it cannot be excluded that different vascular beds remodel in different gradients, with the most prominent changes in the uterine circulation and minor, undetectable alterations in the subcutaneous vascular bed. Thus, it is possible that signs of remodeling are found in other vascular beds in preeclampsia, as has been reported in the omental vascular bed by Aalkjaer et al. [150].

Second, lumen diameter and wall thickness are all highly dependent on the branching level of the vascular tree, where the studied vessel has been taken from [301]. However, wall thickness to lumen ratio of small vessels has been reported to be a highly reproducible morphometric parameter even in small groups of patients [301-303]. We tried to include vessels of roughly the same diameter from women in all groups in this study. Still, we can not exclude the possibility that a systematic sampling error has been introduced, due to differences in the vascular architecture of the resistance vasculature between women with preeclampsia, normal pregnant and nonpregnant women [301,304].

Third, the absence of tethering to surrounding tissue and the lack of effects of perivascular tissue pressure results in the measurement of a greater diameter than is probably present in these arteries in vivo. Furthermore, when transmural pressure is increased vessels not only distend, but also elongate, which could result in a decreased wall thickness to lumen ratio [305]. Whether such elongation also occurs in vivo or whether surrounding tissue prevents this is unclear. Furthermore, it is possible that vessels from different patient groups respond in different ways to the applied in vitro conditions.

Fourth, we assumed that the MMP's circulating in the plasma reflect spill over of the enzymes as a result of their raised levels in the vascular wall during ongoing remodeling. A direct relation between vascular wall and plasma concentrations of these enzymes has however not yet been demonstrated. There are other possible sources of the MMP's in blood, such as the placenta. Placental production of MMP's has been shown to be involved in early placentation [306,307],...
and thus could be involved in the remodeling of the uteroplacental vasculature. To our knowledge no measurements of vascular wall MMP levels have been performed in pregnancy and preeclampsia.

One previous study investigated MMP-2 and MMP-9 concentrations in plasma from women with preeclampsia and normal pregnant women by zymography [308]. Those authors reported that MMP-2 concentrations were three times higher in the blood of women with preeclampsia. No MMP-9 was detected [308]. In that study, however, samples with equal protein loads were loaded on the gel instead of a standard amount of plasma. Since protein concentrations, in specific albumin, are substantially decreased in the blood of women with preeclampsia, larger volumes of plasma from women with preeclampsia were probably loaded onto the gel, possibly explaining why increased MMP-2 concentrations were found in that study and not in ours. The fact that MMP-9 was undetectable in that study can probably be explained by underloading.

Altogether, it seems most important that techniques are developed that allow longitudinal \textit{in vivo} observations of the structure of the same part of the resistance vasculature throughout normal pregnancy and upon development of preeclampsia. This type of longitudinal \textit{in vivo} measurement is possible for monitoring of large arteries (echo wall-tracking [309] or magnetic resonance imaging [310]) and capillaries (OPS imaging and intravital microscopy or capillaroscopy [311]), although these techniques, to our knowledge, have not yet been used for longitudinal monitoring of vascular structure. Hopefully, future studies will indicate whether remodeling is present in these large and small vessels in pregnancy and preeclampsia. Techniques for \textit{in vivo} monitoring of resistance vasculature structure remain to be developed, since current techniques are not applicable for this vasculature mainly due to resolution problems.

In conclusion, we found no direct evidence for vascular remodeling in the isolated subcutaneous arteries in pregnancy and preeclampsia. However, the increased activity of MMP-9 in plasma in pregnancy and preeclampsia could reflect ongoing matrix degradation and thus vascular remodeling in other vascular beds. Measurements in other vascular beds and longitudinal \textit{in vivo} observations are needed to provide evidence whether the process of vascular remodeling is involved in pregnancy and preeclampsia.