Vascular damage and dysfunction in hypertensive emergencies
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Syndecan-1 and glycosaminoglycans in pre-eclamptic and normotensive pregnancies


MANUSCRIPT IN PREPARATION
ABSTRACT

Background: Scavenging of vascular endothelial growth factor (VEGF) by its soluble receptor sFLT-1 is linked to blood pressure (BP) elevation in pre-eclampsia. Syndecan-1 and its associated glycosaminoglycans (GAGs) are essential mediators of VEGF signaling. We aimed to assess whether plasma syndecan-1 and GAGs are altered in pre-eclampsia and contribute to sFLT-1-induced BP elevation.

Methods: Pre-eclamptic and normotensive pregnant women were recruited from the department of obstetrics at the Academic Medical Center in Amsterdam, The Netherlands. Plasma syndecan-1 and GAGs were measured using ELISA and HPLC-MS/MS respectively. In addition, we studied the effect of sFLT-1 on BP and contractile responses in syndecan-1 deficient mice and wild type controls.

Results: In total 125 pregnant women were included, 65 with pre-eclampsia and 60 normotensive controls. Plasma syndecan-1 was similar in pre-eclamptic (553 ng/ml, IQR:309-805) and normotensive women (551 ng/ml, IQR:307-920, \(p=0.96\)). Syndecan-1 was inversely correlated with BP in pre-eclampsia (\(r=0.29, p=0.02\)), but not in normotensives. Plasma dermatan sulphate (DS) was higher in pre-eclamptic women (126 ± 55 ng/ml vs. 74 ± 38 ng/ml \(p=0.01\)), while keratan sulphate was lower compared to normotensives (549 ± 107 ng/ml vs. 781 ± 293 ng/ml, \(p=0.01\)). Heparan sulphate was similar in both groups (1697 ± 824 ng/ml vs. 1401 ± 751 ng/ml, \(p=0.36\)). DS was inversely correlated with BP in pre-eclamptic women (\(r=0.62, p=0.02\)), but not in normotensives. In mice, baseline mean arterial BP was similar in syndecan-1 -/- (75±2 mmHg) and controls (75±1 mmHg, \(p=0.95\)). Treatment with sFlt-1 augmented BP in wild types (86±4 mmHg, \(N=12, p<0.01\)) but not in syndecan-1 -/- mice (78±3 mmHg, \(N=8, p=0.46\)).

Conclusions: Circulating syndecan-1 is similar in pre-eclamptic and normotensive pregnancy. Syndecan-1 is inversely correlated with BP in women with pre-eclampsia, but absence of syndecan-1 prevents sFlt-1-induced BP elevation in mice. DS is elevated in pre-eclampsia and strongly correlated with both syndecan-1 and BP, indicating that alterations in the interplay between syndecan-1 and DS may contribute to sFlt-1 induced BP elevation in pre-eclampsia.
INTRODUCTION

Pre-eclampsia is an important cause of maternal and fetal morbidity and mortality. Scavenging of Vascular Endothelial Growth Factor (VEGF) in the maternal circulation by its soluble receptor fms-related tyrosine kinase-1 (sFLT-1) contributes to endothelial dysfunction, hypertension and proteinuria in women with pre-eclampsia. Excess sFLT-1 is produced by the placenta, possibly in response to hypoxia that results from inadequate placentation.

VEGF signalling is facilitated by the endothelial glycocalyx, which is composed of a complex network of membrane-bound proteoglycans and attached negatively-charged glycosaminoglycans (GAGs) covering the vascular wall. Situated in direct contact with flowing blood, the endothelial glycocalyx regulates various vascular functions such as permeability, leucocyte adhesion, coagulation and vascular tone by mediating shear-dependent nitric oxide (NO) release. Syndecans, a family of proteoglycans, may carry several GAGs, including heparan sulphate (HS) and dermatan sulphate (DS), which determine the signaling function of these proteoglycans. Syndecan-1 is the most abundantly expressed syndecan family member on the endothelium. Syndecan-1 has been shown to regulate VEGF signalling by formation of a complex with VEGFR-2, thereby modulating VEGF-induced motility and migration of endothelial cells. HS chains mediate binding of VEGF to VEGFR-2 by acting as a co-receptor, and depletion of endothelial cell surface HS results in reduced phosphorylation of VEGFR-2. HS also possess a binding domain for sFLT-1, which may serve as reservoir and limit excess placental sFLT-1 release into the circulation. Placental syndecan-1 expression has been shown to be diminished in pre-eclampsia, and reduced circulating syndecan-1 has recently been linked to blood pressure (BP) elevation in women with pre-eclampsia. We hypothesized that the interaction between syndecan-1 and its associated GAGs is pivotal for VEGF signaling and synergistically contribute to sFLT-1-induced BP elevation. In the present study, we assessed whether circulating amounts of syndecan-1 and associated GAGs are altered in pre-eclampsia compared to normotensive pregnancies and whether syndecan-1 deficient mice have a differential BP response to sFLT-1 compared to wild type controls.

METHODS

Study population
Clinical data and plasma samples for analysis of syndecan-1 were obtained from participants in the Pre-eclampsia And Non pre-eclampsia DAtabase (PANDA) program of the department of obstetrics of the Academic Medical Center in Amsterdam, The Netherlands as described previously. Blood samples were drawn prior to delivery in all participating women and
plasma was immediately stored after centrifugation at -80°C. In a small subset of patients, blood was also drawn at 3 months postpartum to assess changes in plasma syndecan-1, HS, DS and keratan sulphate (KS) after pregnancy. We assessed whether syndecan-1 and GAGs were correlated with clinical parameters relevant for pre-eclampsia including BP, proteinuria, platelet count, liver enzymes, lactate dehydrogenase and coagulation. Definitions and clinical criteria used in the biobank program have been published previously. Briefly, pre-eclampsia was defined by systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg recorded on two occasions at least 4 hours apart, after 20 weeks’ gestation in a previously normotensive woman combined with new-onset proteinuria with urinary protein excretion ≥ 300 mg/24-hours. BP was measured manually in the sitting position at the right upper arm using an aneroid sphygmomanometer. Diastolic BP was determined at Korotkoff sound V. Birth weight percentiles were assessed according to the local Dutch birth weight percentiles. The appropriate chart was chosen based on parity and gender of the baby. (http://www.perinatreg.nl/). Small for gestational age was defined as birth weight below 10th percentile. HELLP syndrome was defined by lactate dehydrogenase ≥ 600 U/L or haptoglobin < 0.2 g/L, aspartate or alanine aminotransferase ≥70 U/L, and platelet count < 100 *10⁹/L. Experiments were carried out in accordance with the declaration of Helsinki after informed consent from the participants was obtained. Experiments were approved by an independent ethics committee.

**Plasma syndecan-1 and glycosaminoglycan analysis**

Maternal plasma syndecan-1 concentrations were measured with a commercially available human syndecan-1 enzyme-linked immunosorbent assay (CD138 ELISA Kit, Diaclone), according to the manufacturers’ instructions. Plasma GAGs (HS, DS and KS) were measured in a subset of participants using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) as previously described in detail. HS, DS, and KS were first enzymatically digested into disaccharide units and then quantified on a Waters Quattro Premier XE (tandem) mass spectrometer (Waters Corporation, Milford, MA, USA) coupled to an Acquity UPLC system (UPLC-MS/MS). According to the nomenclature by Lawrence et al, HS was represented by the sum of disaccharides D0A0, D0S0, D0A6 and D2A0, D0S6 and D2S0, DS was represented by D0a4 and D0a10 and KS was represented by the sum of disaccharides g0A6 and G6A6. All samples were digested and analyzed in triplicate.

**Effect of sFlt-1 on blood pressure in wild type and syndecan-1 deficient mice**

To investigate the role of syndecan-1 signaling in sFlt-1-induced BP elevation, we compared BP response *in vivo* and isometric tension measurements *ex vivo* in isolated arteries of syndecan-1 deficient mice and wild type controls. All experimental procedures including housing conditions, BP measurement and isometric tension measurements have been
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described in detail previously. Briefly, adult (12-14 weeks old) male C57/BL6N mice (Charles River, Maastricht, The Netherlands) and syndecan-1 -/- mice on a C57/BL6N background were individually housed in a temperature controlled room with a 12:12 light-dark cycle with food and water *ad libitum*. After acclimatisation, mice were anesthetized for implantation of osmotic minipumps (Alzet, California USA). The pumps were filled with either vehicle (PBS) or sFlt-1 (Creative Biomart, New York, USA, Catalog no: Flt1-1785M) for continuous 0.5µl/h compound release (equals 500ng/h sFlt-1) during 2 weeks. During treatment, BP was recorded at fixed time points using the non-invasive tail cuff BP measurement system, according to the previously described protocol (CODA™ system Kent Scientific Corporation, CT, USA). Animals were trained during one week prior to the experiment initiation to reduce adverse stress responses to the BP measurements. After 2 weeks, mice were euthanized and carotid arteries were isolated for isometric analysis of vasomotor tone. All experimental procedures were approved by the Animal Ethics Committee of the Academic Medical Center, Amsterdam, The Netherlands.

**Wire-myograph analyses**

Carotid arteries were isolated and immediately placed in Krebs-Henseleit buffer (pH 7.4; in mM: 118.5 NaCl, 4.7 KCl, 25.0 NaHCO₃, 1.2 MgSO₄, 1.8 CaCl₂, 1.1 KH₂PO₄ and 5.6 glucose) for connective tissue removal. Artery segments of 2 mm were then mounted into a multichannel wire myograph for isometric tension measurements. Vessel segments were incubated with carbogen (95% O₂, 5% CO₂) aerated Krebs-Henseleit buffer at a temperature of 37° C. The buffer was replaced every 15 minutes during the experiment. In all experiments the segments were first contracted with high K⁺-containing Krebs buffer (pH 7.4; in mM: 23.2 NaCl, 100 KCl, 25 NaHCO₃, 1.2 MgSO₄, 1.8 CaCl₂, 1.1 KH₂PO₄ and 5.6 glucose). After 30 minutes washout a concentration-response-curve (CRC) of the α1-adrenoceptor agonist phenylephrine was generated in half-log concentration increments (1 nM – 0.1 µM). Contraction on phenylephrine was immediately followed by a methacholine CRC (1 nM- 1 µM) to assess endothelium-dependent vasodilatation. Next after 15 minutes of washout a high K⁺ Krebs-induced CRC (5 mM – 100 mM) was generated. Finally an ET-1 CRC (0.1 nM – 0.3 µM) was generated in half-log concentration increments. Contractile force of carotid artery segments is expressed in milinewton (mN).

**Statistical analysis**

Continuous clinical variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) for variables with a skewed distribution. Categorical data are expressed as number and percentages. Between group differences were assessed by t-test for parametric and Mann–Whitney U test for non-parametric distributions. Chi-square statistics were used for categorical variables. Comparison of plasma syndecan-1 and GAGs
among pre-eclamptic women with and without HELLP and normotensives was carried out by Kruskall-Wallis test and one-way ANOVA respectively. Linear regression analysis was used to assess the correlation of syndecan-1 and GAGs with clinical parameters. For animal experiments, BP and isometric tension measurements are presented as means ± standard error of the mean (SEM). Independent t-test was used to compare wild types and syndecan-1 -/- mice. Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 19.0, Inc. Chicago, Illinois, USA). P-values were considered to indicate a significant difference if p<0.05.

RESULTS

Clinical characteristics of participants
In total 125 pregnant women were included in this study, 65 pre-eclamptic and 60 normotensive. Clinical characteristics of pregnant women with and without pre-eclampsia are summarized in Table 1. HELLP syndrome was present in 18 (28%) women with pre-eclampsia. Magnesiumsulphate (MgSO4) was administered to 26 (40%) pre-eclamptic women. Antihypertensive treatment was administered to 47 (72%) women with pre-eclampsia, 15 (32%) of which received 1 antihypertensive agent, 20 (43%) received 2 antihypertensive agents and treatment with 3 or more different antihypertensive drugs was required in 12 (26%) pre-eclamptic women. Of all women treated with antihypertensives, 40 (85%) received a calcium-antagonist (nifedipine OROS), 15 (32%) received a combined α1 and β-blocking agent (labetalol), 36 (77%) received a central α1 agonist (methyldopa) and 2 (4%) were treated with a selective serotonin 5-HT2-antagonist (ketanserin). Eleven (17%) women with pre-eclampsia were not treated with any type of antihypertensive medication, of these women, one received MgSO4 prior to delivery. A calcium antagonist (nifedipine OROS) was administered to 9 (15%) normotensive women as a tocolytic agent to delay imminent preterm labour.
Table 1. Clinical characteristics with comparison of normotensive pregnant women and women with pre-eclampsia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-eclampsia</th>
<th>Normotensive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, N</td>
<td>65</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>31±5</td>
<td>29±6</td>
<td>0.06</td>
</tr>
<tr>
<td>Caucasian*</td>
<td>27 (51%)</td>
<td>27 (60%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Body Mass Index, kg/m2</td>
<td>27±6</td>
<td>25±7</td>
<td>0.10</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>154±20</td>
<td>117±14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>96±11</td>
<td>68±11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proteinuria, g/24hrs</td>
<td>1.50 [0.57-4.43]</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Platelet count, x10⁹/L</td>
<td>148±84</td>
<td>234±55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>300 [239-551]</td>
<td>176 [136-213]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nulliparous**</td>
<td>35 (57%)</td>
<td>34 (58%)</td>
<td>0.98</td>
</tr>
<tr>
<td>Gestational age at delivery, days</td>
<td>236±27</td>
<td>239±27</td>
<td>0.47</td>
</tr>
<tr>
<td>Birth weight, grams</td>
<td>1914±832</td>
<td>2292±876</td>
<td>0.02</td>
</tr>
<tr>
<td>Small for gestational age§</td>
<td>7 (15%)</td>
<td>10 (19%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Antenatal steroids†</td>
<td>18 (40%)</td>
<td>20 (42%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Delivery by caesarean section#</td>
<td>35 (63%)</td>
<td>11 (19%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Numbers represent mean ± standard deviation, median with [interquartile range], or number of subjects with percentages. *Data on ethnicity missing for 12 women with pre-eclampsia and for 15 normotensive women. **Data missing for 4 women with pre-eclampsia and 1 normotensive woman. §Data missing for 18 pre-eclamptic and 8 normotensive women. †Data missing for 20 women with pre-eclampsia and 12 normotensive women. #Data missing for 9 pre-eclamptic women and 2 normotensive women.

Syndecan-1 in pre-eclamptic and normotensive pregnancy and at 3 months postpartum

Plasma syndecan-1 concentration was similar in pre-eclamptic and normotensive women prior to delivery and drastically decreased in both groups at 3 months postpartum (Table 2). Comparison of median plasma syndecan-1 among normotensive pregnant women (551 ng/ml, IQR: 307-920) and pre-eclamptic women with (644 ng/ml, IQR: 286-919) and without HELLP syndrome (447 ng/ml, IQR: 267-734) also showed no significant differences (p=0.41). There was no significant correlation between syndecan-1 and gestational age within the included gestational age range of 173 to 276 days, but a borderline-significant trend was observed in women with pre-eclampsia (r=0.24, p=0.07).

Table 2. Plasma syndecan-1 during normotensive and pre-eclamptic pregnancy and at 3 months postpartum

<table>
<thead>
<tr>
<th>Syndecan-1 (ng/ml)</th>
<th>Pre-eclampsia</th>
<th>Normotensive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum</td>
<td>17 [15-18]</td>
<td>15 [10-17]</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Numbers represent median and [interquartile range]. Postpartum syndecan-1 was determined in 5 previously normotensive pregnant women and 4 previously pre-eclamptic women.
Glycosaminoglycans in pre-eclamptic and normotensive pregnancy and at 3 months postpartum

Prior to delivery, DS was higher in pre-eclamptic women (126 ± 55 ng/ml, N=14) compared to normotensive pregnant women (74 ± 38 ng/ml, N=11, \( p = 0.01 \)). At 3 months postpartum, DS was similar in former pre-eclamptic (28 ± 3 ng/ml, N=3) and former normotensive pregnant women (30 ± 29 ng/ml, N=2, \( p = 0.91 \)). KS was lower in pre-eclamptic women (549 ± 107 ng/ml, N=14) compared to normotensive pregnant women (781 ± 293 ng/ml, N=11, \( p = 0.01 \)). Postpartum, KS was similar in previously pre-eclamptic women (883 ± 105 ng/ml, N=3) and previously normotensive women (680 ± 48 ng/ml, N=2, \( p = 0.62 \)). HS was similar in pre-eclamptic women (1697 ± 824 ng/ml, N=11) and normotensive pregnant women (1401 ± 751 ng/ml, N=14, \( p = 0.36 \)). HS was drastically decreased at 3 months postpartum in previously pre-eclamptic women (94 ± 29 ng/ml, N=3) and normotensives (58 ± 23 ng/ml, N=2, Figure 1). HS, DS and KS were not correlated with gestational age within the included range of 190 to 276 days gestation.

Figure 1. Plasma glycosaminoglycans during normotensive and pre-eclamptic pregnancy and at 3 months postpartum

Bars represent mean with standard deviation. Plasma heparan sulphate (HS), dermatan sulphate (DS) and keratan sulphate (KS) are compared among normotensive pregnant women (NT, N=11) and pre-eclamptic women (PE, N=14). Postpartum values were measured in 3 pre-eclamptic women and 2 normotensive women.* Indicates \( p < 0.01 \), # indicates \( p < 0.05 \).
Syndecan-1 is inversely correlated with BP in pre-eclamptic women

Syndecan-1 was inversely correlated with systolic BP in pre-eclamptic women, showing higher BP values with decreasing plasma concentration of syndecan-1 (Figure 2A). This association was also evident from the number of required antihypertensives, which showed lower syndecan-1 values in women receiving more antihypertensive medication (Figure 2B). Syndecan-1 was not correlated with systolic BP in normotensive pregnant women ($r=0.05$, $p=0.75$). There was no correlation between syndecan-1 and other clinical parameters including proteinuria, platelet count, lactate dehydrogenase, liver enzymes and birth weight percentiles in women with pre-eclampsia.

![Figure 2. Syndecan-1 is correlated with blood pressure and antihypertensive medication in women with pre-eclampsia](image)

Correlation of plasma syndecan-1 with systolic blood pressure showing increasing blood pressure values with lower syndecan-1 (A). Plasma syndecan-1 among pre-eclamptic women categorized by number of received antihypertensive medication (B). Median syndecan-1 decreases with increasing number of antihypertensives. Each symbol represents an individual patient with pre-eclampsia, the horizontal line represents the median.

Dermatan sulphate is strongly correlated with syndecan-1 and BP in pre-eclamptic women

Plasma HS and DS were strongly correlated with circulating syndecan-1 in women with pre-eclampsia (Figure 3A and 3B). DS was inversely correlated with men arterial BP in women with pre-eclampsia (Figure 3C), but not in normotensive pregnant women ($r=0.46$, $p=0.25$). DS was strongly correlated with activated partial thromboplastin time (aPTT) in women with pre-eclampsia (Figure 3D). DS was not correlated with other clinical parameters including proteinuria, platelet count, lactate dehydrogenase, liver enzymes and birth weight. HS sulphate and KS were not correlated with any pre-eclampsia-related clinical parameter.
Correlation of plasma heparan sulphate (A) and dermatan sulphate (B) with syndecan-1 in pre-eclampsia. Dermatan was inversely correlated with mean arterial blood pressure (C) and activated partial thromboplastin time (aPTT) in women pre-eclampsia (D). Correlations were absent in normotensives.

sFlt-1 elevated blood pressure in wild types but not in syndecan-1 +/- mice
To assess whether syndecan-1 contributes to sFlt-1-BP elevation, we treated syndecan-1 deficient mice and wild type controls with sFlt-1 or vehicle (PBS) for a period of two weeks. Baseline mean arterial blood pressure (MAP) was similar in syndecan-1 +/- (75 ± 2 mmHg, N=16-32, p=0.95) and wild type mice (75 ± 1 mmHg, N=16-32, p=0.95). In wild type mice, sFlt-1 infusion induced a MAP elevation to 86 ± 4 mmHg, N=12, p<0.01), while MAP remained unchanged after vehicle-treatment (74 ± 3 mmHg, N=11, p=0.55). In contrast, MAP of syndecan-1 +/- mice, remained unchanged after sFlt-1 (78 ± 3, N=8) or vehicle (75 ± 2 mmHg, N=8) treatment (p=0.66, Figure 4).
Effect of sFlt-1 or vehicle infusion during two weeks on mean arterial blood pressure (MAP) of syndecan-1 deficient mice and wild type controls. Syndecan-1 deficient mice had similar MAP at baseline (N=16) and after treatment with sFlt-1 (N=8) and vehicle (n=8). Treatment with sFlt-1 significantly elevated MAP in wild type control mice (N=12) compared to baseline (N=32) and vehicle (N=11) treatment. Bars represent mean ± SEM, ns indicates not-significant, * indicates p<0.01.

Isometric tension measurements in syndecan-1 -/- and wild type mice

In isolated carotid artery segments of wild type mice, maximal endothelin-1-induced vasoconstriction was increased after sFlt-1 treatment (1.2 ± 0.3 mN, N=6) compared to vehicle treatment (0.5 ± 0.1 mN, N=6, p=0.03). In syndecan-1 -/- mice, endothelin-1-induced vasoconstriction was similar after sFlt-1 (0.59 ± 0.10 mN, N=5) and vehicle treatment (0.64 ± 0.06 mN, N=4, p=0.68, Figure 5A). In wild types, maximal metacholine-induced vasodilatation (after preconstriction with phenylephrine) was decreased after sFlt-1 treatment (72 ± 4%, N=14), compared to vehicle treatment (83 ± 3%, N=14, p=0.04). In syndecan-1 -/- mice, maximal metacholine-induced vasodilatation was similar after sFlt-1 treatment (89 ± 3%, N=5) and vehicle treatment (86 ± 4%, N=4, p=0.58, Figure 5B). Maximal metacholine-induced vasodilatation was similar in vehicle-treated syndecan-1 -/- mice an vehicle-treated wild types (p=0.62), but was significantly higher in sFlt-1-treated syndecan-1 -/- mice compared to sFlt-1-treated wild types (p=0.04).
Figure 5. Endothelin-1-induced contraction in sFlt-1 and vehicle-treated on syndecan-1 -/- mice and wild type controls

(A) In wild type mice, sFlt-1 infusion significantly augmented maximal endothelin-1-induced vasoconstriction of carotid artery segments ex vivo compared to vehicle treatment (N=6 in each group, \( p = 0.03 \)). In syndecan-1 deficient mice, endothelin-1-induced vasoconstriction was similar after sFlt-1 and vehicle treatment (N=5-4, \( p = 0.68 \)).

(B) In wild types, maximal metacholine-induced vasodilatation (after preconstriction with phenylephrine) was decreased in sFlt-1-treated compared to vehicle-treated mice (N=14 in each group, \( p = 0.04 \)). In syndecan-1 deficient mice, maximal metacholine-induced vasodilatation was similar after sFlt-1 and vehicle treatment (N=5-4, \( p = 0.58 \)).
DISCUSSION

In the present study we show that plasma syndecan-1 levels are similar in plasma of pre-eclamptic and normotensive pregnant women. Even in pre-eclamptic women with HELLP syndrome, syndecan-1 was not elevated in contrast to previous observations. Circulating GAGs were however significantly altered in pre-eclampsia and strongly correlate with syndecan-1 in pre-eclamptic women, but not in normotensive controls. Both syndecan-1 and DS were inversely correlated with BP, suggesting that alterations in endothelial glycocalyx composition may contribute to BP elevation in pre-eclampsia.

Third trimester plasma syndecan-1 has previously been shown to be decreased in a total of 27 pre-eclamptic pregnancies compared to a similar amount of uncomplicated control pregnancies. We could not confirm this observation in a larger group of women with pre-eclampsia. In comparison with the most recent study by Gandley et al. and their online data publication, syndecan-1 appeared to be similar in pre-eclamptic women in both studies. In fact, the discrepancy between the studies is explained by significantly higher syndecan-1 values among controls in the study by Gandley et al. compared to normotensive controls in our study. While all controls in the prior study had uncomplicated pregnancies, normotensive controls in our study included women with preterm labour of unknown cause and women with small for gestational age (SGA) neonates. Pre-eclamptic women with SGA neonates were shown to have lower syndecan-1 in the study by Gandley et al., possibly explaining the lower syndecan-1 among normotensive controls in the present study. Indeed, comparison of syndecan-1 among women with and without SGA neonates within in each group, showed lower syndecan-1 in those with SGA neonates, yet not significantly.

Although our study was not designed to systematically address syndecan-1 in different stages of pregnancy, we could confirm the massive rise of plasma syndecan-1 during both normotensive and pre-eclamptic pregnancy (data not shown) and subsequent decrease at 3 months postpartum. This pattern supports the idea that excess circulating syndecan-1 originates mainly from the placenta. Syndecan-1 is expressed on syncytiotrophoblast cells, which are in direct contact with maternal blood. Shedding of syndecan-1 from the syncytiotrophoblast would likely increase maternal plasma syndecan-1 during pregnancy. Placental expression of syndecan-1 has been shown to be reduced in pre-eclampsia by multiple independent studies, possibly explaining the reduced plasma syndecan-1 at mid-pregnancy. The endothelium could be an alternative source of syndecan-1, which is shed from the endothelial glycocalyx upon damage to the endothelium. The inverse correlation of syndecan-1 with BP and the observation of similar or lower plasma syndecan-1 values in pre-eclamptics women compared to normotensive controls, indicate that hypertension-induced endothelial glycocalyx shedding is not the main source of circulating syndecan-1 in pre-eclampsia. However, the actual contribution of endothelium-derived syndecan-1 to the total circulating amount is unknown.
In our syndecan-1 knockout model we were able to elaborate some more on the observed correlations between syndecan-1 and BP in pre-eclampsia, which was also demonstrated by Gandley et al. We showed that sFlt-1-induced BP elevation was absent in syndecan-1 deficient mice. We have previously shown that increased endothelin-1-mediated vasoconstriction and decreased metacholine-induced vasodilatation contributed to sFlt-1-induced BP elevation in wild types. These ex vivo effects on contractility were absent in syndecan-1 -/- mice, suggesting that deficiency of syndecan-1 is essential for sFlt-1-induced hypertension. The potential effect of syndecan-1 on BP seems to be mediated through its attached GAGs, which are generally not affected in syndecan-1 deficient mice. Indeed administration of sulodexide, a mixture of GAGs containing 20% DS, significantly lowers BP in hypertensive and normotensive individuals, possibly by mediating binding of circulating VEGF to the endothelium. This hypothesis is supported by the positive correlation of syndecan-1 with HS and DS and the strong inverse correlation of DS with BP in pre-eclamptic women. HS and DS were drastically increased in both pre-eclamptic and normotensive pregnancy and were much lower postpartum, suggesting that excess GAGs predominantly originate from the placenta. However, we cannot rule out the possibility that the endothelium contributes to significantly elevated plasma DS. The syncytiotrophoblast, which is in direct contact with maternal blood has been shown to be devoid of DS, providing support to an endothelial origin of excess DS. In addition, endocan, an endothelial-cell specific soluble DS proteoglycan, has been shown to be elevated in women with pre-eclampsia. Plasma endocan was shown to be positively correlated with circulating sFLT-1 in pre-eclampsia, potentially explaining the observed inverse correlation of DS with BP in the present study. In addition to interaction with sFLT-1, DS is able to bind transforming growth factor-β (TGF-β). Disruption of TGF-β signaling has been linked to endothelial dysfunction and decreased nitric-oxide availability in pre-eclampsia. TGF-β signaling is modulated by soluble endoglin, which is also positively correlated with the soluble DS proteoglycan endocan in pre-eclampsia. Although merely speculative, elevated circulating DS levels may also be related to BP via interaction with TGF-β. Further support for an active role of elevated DS in pre-eclampsia is provided by our observation that DS was strongly correlated with aPTT. Accordingly, DS has been previously been identified as a major determinant of coagulation in the placenta via activation of the thrombin inhibitor heparin cofactor II within fetal vessel walls. In conclusion, circulating syndecan-1 is similar in pre-eclamptic and normotensive pregnancy and most likely originates from the placenta. Syndecan-1 is inversely correlated with BP and the need for antihypertensive treatment in women with pre-eclampsia. Treatment of syndecan-1 deficient mice with sFlt-1 did not augment blood pressure, suggesting that syndecan-1 is pivotal for sFlt-1-induced BP elevation. DS was significantly elevated in pre-eclampsia and inversely correlated with syndecan-1 and BP, suggesting that the association of syndecan-1 with BP in pre-eclampsia might be mediated by DS. Administration DS has
been shown to lower BP in hypertensive and normotensive individuals, providing additional support for an active role of DS in elevating BP in women with pre-eclampsia.
REFERENCES


