Plasma soluble endothelial selectin is elevated in women with pre-eclampsia

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The study was conducted to determine whether altered plasma concentrations of soluble selectins are involved in the pathogenesis of pre-eclampsia. Maternal plasma samples were collected from 20 patients with pre-eclampsia, and from 20 matched normotensive patients with uncomplicated pregnancies. Samples were assayed for soluble endothelial selectin (sES), platelet selectin (sPS) and leukocyte selectin (sLS) by specific enzyme-linked immunosorbent assay. The three soluble selectins were detectable in the plasma of all pre-eclamptic and control patients. The mean plasma concentrations of sPS and sLS were comparable between the groups. However, the mean plasma concentration of sES was significantly higher in the pre-eclamptic group compared with the control group (61 ng/ml ± 30 ng/ml compared with 40 ng/ml ± 17 ng/ml; P < 0.01). The selective increased plasma concentrations of sES in patients with pre-eclampsia provide specific evidence for endothelial activation and may reflect distinct pathways for neutrophil activation in pre-eclampsia.

Key words: endothelial activation/neutrophil activation/platelet activation/pre-eclampsia/soluble selectins

Introduction

Despite intensive efforts to delineate the pathophysiology of pre-eclampsia, neither a specific cause nor a pathogenesis has been identified (Zeeman and Decker, 1992; Roberts and Redman, 1993; Taylor, 1997). Recent studies suggest that the basic pathophysiological abnormality is endothelial cell dysfunction, as suggested by elevated concentrations of some nonspecific markers (Taylor et al., 1990; Zeeman and Decker, 1992; Roberts and Redman, 1993; Deng et al., 1994; Taylor, 1997). A great deal of evidence suggests that pre-eclampsia may be associated with inflammatory and immune responses (Zeeman and Decker, 1992; Roberts and Redman, 1993; Taylor, 1997) that, in addition to endothelial activation, entails activation of the leukocytes and platelets (Haeger et al., 1992; Zeeman and Decker, 1992; Roberts and Redman, 1993; Abdul Halim et al., 1996; Taylor, 1997). The selectins, a group of cell adhesion molecules, are surface glycoproteins which interact with carbohydrate ligands on leukocytes and endothelial cells and mediate the adhesion of leukocytes and platelets to activated vascular endothelium. This interaction leads to cellular extravasation and the development of immune and inflammatory responses (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver 1994; Tedder et al., 1995; Marik and Lo, 1996). The selectins comprise three molecules: endothelial selectin (ES), which is expressed exclusively on endothelial cells following their activation, platelet selectin (PS), expressed on endothelial and platelet cells upon activation of these cells, and leukocyte selectin (LS), which is expressed constantly in a wide variety of leukocytes, and plays a role in the migration of lymphocytes and neutrophils into acute and chronic inflammatory sites (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver, 1994; Tedder et al., 1995; Marik and Lo, 1996).

The selectin molecules also exist in soluble isoforms (sES, sPS, sLS) that are shed during inflammatory and immune reactions and may thus reflect disease activity (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver, 1994; Tedder et al., 1995; Marik and Lo, 1996). This study was conducted to determine whether altered concentrations of sES, sPS and sLS may be involved in the pathogenesis of pre-eclampsia.

Materials and methods

The study was approved by the Institutional Review Board and informed consent was obtained from each patient. The study group comprised 20 women with pre-eclampsia. The diagnosis of pre-eclampsia was determined if the patients met the criteria of the American College of Obstetricians and Gynecologists for pre-eclampsia (American College of Obstetricians and Gynecologists, 1996), and was defined as persistent blood pressure of ≥140/90 with proteinuria ≥100 mg/dl by urine analysis or ≥300 mg/24 h. Cases complicated by chronic hypertension, diabetes, chronic renal disease, preterm labour, premature rupture of membranes, and autoimmune disorders were not included in the study.

Healthy patients (n = 20), matched with the pre-eclamptic patients for age, gestational age and parity, comprised the control group. None developed pre-eclampsia, or other pregnancy complications. Blood samples were collected on hospital admission from the patients with pre-eclampsia, and during a routine outpatient prenatal visit for the controls. Samples were collected prior to the onset of labour, induction of labour or any medical intervention. All blood specimens were centrifuged at 1000 g for 10 min and the plasma was then stored in aliquots at −70°C until assayed collectively by an investigator blinded to patient assignment.

Soluble ES, PS and LS were measured in duplicate by commercial
Table I. Clinical data for study and control groups. Values are presented as mean ± SD, except where shown.

<table>
<thead>
<tr>
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<th>Study group (n = 20)</th>
<th>Control group (n = 20)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.1 ± 5.8</td>
<td>28.7 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>36.6 ± 3.6</td>
<td>36.6 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>No. (%) of nulliparous</td>
<td>15 (75)</td>
<td>15 (75)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>107 ± 8</td>
<td>75 ± 4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>171 ± 20</td>
<td>117 ± 6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NS = not significant.

enzyme-linked immunosorbent assay (ELISA) (human soluble ES, PS and LS; R&D Systems, Minneapolis, MI, USA) specific for each molecule. These assays employ the quantitative sandwich immunosorbent technique that involves the simultaneous reaction of the measured molecules with two murine monoclonal antibodies specifically directed against two different epitopes on human soluble ES, PS and LS. One of these antibodies is conjugated to the enzyme horseradish peroxidase (HRP). After removal of unbound material by aspiration and washing, the amount bound to the well is detected by reaction with a substrate specific for the enzyme that yields a coloured product proportional to the amount of conjugate. The coloured product is quantified photometrically and the concentration is calculated by analysing standards of known concentration.

These monoclonal antibodies are specific and cross-reactivity to other selectins or cell adhesion molecules were not observed. The inter-assay and intra-assay coefficients of variation for ES were: <9% and 5% respectively, for PS <9.9% and 5.6% respectively, and for LS <7% and 4.7% respectively. The sensitivity of the assays for plasma soluble ES, PS and LS was <0.1 ng/ml, <0.5 ng/ml and <0.3 ng/ml respectively.

Demographic and clinical data were compared using Student’s t-test and the χ² test. Soluble selectin concentrations were compared using Student’s t-test. Associations between selectin concentrations and other parameters were determined by the Pearson correlation test. P ≤ 0.05 was considered to be statistically significant.

Results

As expected from the recruitment criteria, the mean maternal age, gestational age and parity were similar for the pre-eclamptic and normal pregnant patients, and are depicted in Table I. The three soluble selectins were detected in the plasma of all pre-eclamptic and control women. The mean plasma concentrations of sPS and sLS were comparable between the study and control groups (103 ng/ml ± 78 ng/ml compared with 87 ng/ml ± 6 ng/ml, and 581 ng/ml ± 139 ng/ml compared with 578 ng/ml ± 105 ng/ml respectively (Figures 1 and 2). However, the mean plasma concentration of sES was significantly higher in the pre-eclamptic group (61 ng/ml ± 30 ng/ml compared 40 ng/ml ± 17 ng/ml, P < 0.01; Figure 3).

Soluble ES was significantly correlated with proteinuria (r = 0.54; P = 0.05), creatinine concentrations (r = 0.54; P = 0.02) and uric acid concentrations (r = 0.56; P = 0.03) (Figure 4). No correlation between selectin concentrations and systolic or diastolic blood pressure was found.

Discussion

The three soluble selectins were detected in the plasma of all patients studied, indicating that soluble selectins are physiologic constituents of plasma during pregnancy. This finding is in accordance with the theory suggested by Schuiling et al. (1997), proposing that normal implantation and pregnancy are physiologically associated with a maternal inflammatory response. Furthermore, soluble glycoconjugates, such as soluble selectins, may participate in placental lymphocyte homing and may abrogate the maternal immune response by blocking the common carbohydrate sequences required for inflammatory and immune processes, thus creating part of the

Figure 1. Plasma soluble platelet selectin (P-selectin) concentrations in women with pre-eclampsia and normotensive pregnant women.

Figure 2. Plasma soluble leukocyte selectin (L-selectin) concentrations in women with pre-eclampsia and normotensive pregnant women.
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Soluble ES concentrations were significantly increased in women with pre-eclampsia, compared to control normotensive patients. This finding is in accordance with data recently reported by Austgulen et al. (1997) and others (Fleckling et al., 1995; Lyall and Greer, 1995). The finding of elevated sES concentrations provided convincing evidence for the notion of endothelial activation in pre-eclampsia, since ES expression is restricted to activated endothelial cells and the raised plasma concentrations of sES represent increased shedding from these activated cells (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver 1994; Tedder et al., 1995; Marik and Lo, 1996). Markers of endothelial activation, such as cellular fibronectin (Deng et al., 1994), von Willebrand factor (Deng et al., 1994), endothelin-1 (Taylor et al., 1990), and thrombomodulin (Zeeman and Decker, 1992; Roberts and Redman, 1993; Taylor, 1997), have also been reported to increase in pre-eclampsia. However, these markers are not produced exclusively by the endothelium. For example, cellular fibronectin is produced by fibroblasts, fat-storing liver cells and synovial cells (Deng et al., 1994), while von Willebrand factor is produced by megakaryocytes (Deng et al., 1994).

ES is not constantly present in endothelial cells, but its expression and shedding are induced upon activation of the endothelium by interleukin-1-β, and tumour necrosis factor (TNF)-α which were reported to be increased in patients with pre-eclampsia (Kupferminc et al., 1994, 1996). This could represent one mechanism for the increased sES concentrations in pre-eclampsia. Another possible explanation for increased sES in pre-eclampsia could be a recently described factor originating from the syncytiotrophoblast that increases the shedding of adhesion molecules in pre-eclampsia and promotes endothelial activation (Smarason et al., 1993). Schuiling et al., (1997), proposed that normal implantation is associated with an inflammatory response and that pre-eclampsia results from a violent, unregulated inflammatory response due to defective implantation. Thus, the increased concentrations of plasma sES in pre-eclampsia may reflect heightened shedding due to the intense inflammatory reaction that accompanies the disease process. Further support for this inflammatory theory is provided by the observation that recombinant sES has been shown to function as a chemo-attractant (Lo et al., 1991) for neutrophils supporting other data for neutrophil activation in pre-eclampsia (Haegar et al., 1992).

The pathogenesis of pre-eclampsia arises from the placenta (Redman, 1991). Adhesion molecules have a crucial role in the process of placental formation and vascular invasion (Redman, 1997). Furthermore, during the process of vascular invasion, the cytotrophoblasts adopt a vascular phenotype by expressing a set of adhesion molecules that simulates the endothelium (Zhou et al., 1997a). Abnormal expression of adhesion molecules may result in aberrant placentation and restricted endovascular invasion (Brosens et al., 1972), culminating clinically as pre-eclampsia (Zhou et al., 1993, 1997b; Redman, 1997). Abnormal concentrations of sES may be a further reflection of this aberrancy.

Figure 3. Plasma soluble endothelial selectin (E-selectin) concentrations in women with pre-eclampsia and normotensive pregnant women.

Figure 4. Correlation between plasma soluble endothelial selectin (soluble ES) concentrations and proteinuria, creatinine and uric acid concentrations. ♦ = creatinine concentrations (µg/l; trendline --); ▲ = uric acid concentrations (µg/dl; trendline -----); ● = proteinuria (semi-quantitative, µg/ml; trendline ......).
Our knowledge regarding selectins and their soluble forms comes mainly from the inflammatory process where endothelial, platelet and leukocyte activation involves all three selectins and consequently results in a rise of their soluble products (Gearing and Newman, 1993). However, we detected a selective increase in sES, rather than in all three soluble selectins. This selective rise in sES may be of similar significance to the reported increase of sES in diabetes and systemic lupus erythematosus, where endothelial cell dysfunction is predominant (Gearing and Newman, 1993). It is therefore possible that different pathological processes are dominated by a selective or preferential activation of different cell types. Nevertheless, increase in sES concentrations could also be secondary to impaired renal function in pre-eclampsia, as sES concentrations correlated with proteinuria, uric acid and creatinine concentrations in pre-eclamptic patients. However, it is not known how soluble selectins are cleared from the circulation, and whether all three are cleared by the same mechanism (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver 1994; Tedder et al., 1995; Marik and Lo, 1996).

Plasma concentrations of sPS were similar in pre-eclamptic and control patients. This finding is in agreement with other authors (Konijnenberg et al., 1996; Krauss et al., 1996), but in contrast to data recently published by Abdul Halim et al. (1996), who reported increased concentrations of sPS in the plasma of 10 patients with pre-eclampsia and 20 patients with eclampsia, compared with 10 control women. Abdul Halim et al. (1996) speculated that their finding of increased sPS supports platelet activation in pre-eclampsia. However, the role of sPS in vivo is not well defined (Gearing and Newman, 1993; Tedder et al., 1995). While PS is involved in platelet–endothelial cell recognition, and contributes to the accumulation of platelets and neutrophils at inflammatory sites (Gearing and Newman, 1993; Marik and Lo, 1996), it has been suggested that sPS plays an inhibitory and protective role by preventing adherence of activated neutrophils and platelets to activated endothelium, and preventing superoxide release from neutrophils (McEver, 1994). Thus, sPS may function as an anti-inflammatory agent, and may not necessarily reflect platelet activation.

Soluble LS has not been studied previously in women with pre-eclampsia. Elevated concentrations of sLS are usually observed in cases of lymphocyte and/or neutrophil activation during acute and chronic inflammatory reactions and the loss of surface LS may be necessary to facilitate leukocyte migration through the endothelium (Lewinsohn et al., 1987; Schleiffenbaum et al., 1992; Gearing and Newman, 1993; McEver 1994; Tedder et al., 1995; Marik and Lo, 1996). Furthermore, it has been indicated that sLS may also act as an inhibitory factor by inhibiting the interaction of lymphocytes and neutrophils with the endothelium (Gearing and Newman, 1993; Tedder et al., 1995).

In the present study, sLS was not increased in women with pre-eclampsia, and accordingly it does not support leukocyte activation in pre-eclampsia. This finding appears to contradict the reported data that suggest neutrophil activation in pre-eclampsia (Haegar et al., 1992), including the reported elevation of sES found in this and other studies (Fleckling et al., 1995; Lyall and Greer, 1995; Austgulen et al., 1997). This contradiction is not easily explained. Nevertheless, both sPS and sLS were reported as possessing anti-inflammatory properties in contrast to sES, which augments inflammation (Lewinsohn et al., 1987; Gearing and Newman, 1993; McEver 1994; Tedder et al., 1995; Marik and Lo, 1996). Thus, we postulate that the selective increase in sES concentrations may cause an imbalance in the inflammatory process in pre-eclampsia, which results in acceleration of the cellular damage, or that this finding may reflect specific pathways for neutrophil activation in pre-eclampsia without eliciting the entire inflammatory process.

References
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