Effects of pre-eclampsia on maternal plasma, cerebrospinal fluid, and umbilical cord urotensin II concentrations: a pilot study

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Background. Urotensin II (UII) is the most potent endogenous vasoconstrictor identified to date. Pre-eclampsia is associated with arteriolar vasospasm but the precise underlying mechanism is uncertain and we hypothesized that UII concentrations might also be elevated. In this study we measured UII concentrations in maternal plasma and cerebrospinal fluid (CSF), and umbilical vein plasma from pre-eclamptic (PET) and normotensive patients undergoing elective Caesarean section under spinal or combined spinal–epidural anaesthesia.

Methods. With LREC approval and informed consent we recruited two groups of 10 patients: control [mean (range) age, 29 (22–43) yr; BMI, 25 (20–32); gestation, 273 (267–281) days; mean arterial pressure (MAP) on day of delivery, 81 (75–96) mm Hg] and PET [age, 34 (22–40) yr; BMI, 25 (21–46); gestation, 253 (203–289) days; MAP on day of delivery, 106 (88–128) mm Hg]. Maternal blood and CSF samples and umbilical vein blood samples were taken. UII was extracted and concentrations measured using a radioimmunoassay.

Results. Two plasma and two CSF samples in the control and two CSF samples in the PET group were below the assay detection limits. There were no differences in maternal plasma or CSF or umbilical vein UII concentrations between the groups. However, there was a small (~40%) but significant increase in cord UII concentrations when compared with paired plasma in the PET group. There was a weak but significant negative correlation ($r = -0.4, P = 0.049$) between cord UII concentrations and gestation in the PET group. In addition, we observed a significant positive correlation between plasma and CSF ($r^2 = 0.57, P = 0.0009, n = 16$), plasma and cord ($r^2 = 0.43, P = 0.0031, n = 18$) and CSF and cord ($r^2 = 0.32, P = 0.022, n = 16$) UII concentrations for the whole data set.

Conclusions. Collectively the data indicate that UII concentrations do not increase in PET compared with controls but, in PET patients, cord UII concentrations are elevated relative to paired plasma samples. Elevated umbilical vein UII concentrations may simply indicate reduced placental viability and possibly UII metabolism as a result of reduced blood flow or possibly that the placenta is producing UII.

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Urotensin II (UII), from the marine goby, was characterized as vasoactive in the 1960s. UII is currently the most potent vasoconstrictor identified, being two orders of magnitude more potent than endothelin-1. In man, the peptide is composed of 11 amino acids with a highly conserved cyclic
hexapeptide sequence between residues 6 and 10. UII produces potent vasoconstriction in some but not all vascular beds with variable efficacy: UII is generally described as an arterial constrictor but relaxation has been observed in some vessels. UII concentrations are increased in cardiac failure, renal failure, diabetes as well as in hypertensive states. UII interacts with a specific G-protein-coupled receptor termed UT, which is localized on vascular smooth muscle, vascular endothelium, and is also found in the central nervous system (for reviews see 2–9).

Pre-eclampsia (PET) is a disease of pregnancy (from about the 20th week of gestation) associated with intense vasospasm, which presents as hypertension, poor organ perfusion affecting the kidney (causing proteinuria and peripheral oedema), the central nervous system, and liver. Coagulopathies and intra-uterine fetal growth restriction are often observed.10–12 The aetiology of pre-eclampsia is the subject of much debate but is generally believed to result from arteriolar vasoconstriction with abnormal invasion of the spiral arteries by cytotrophoblast cells12 and hence placental dysfunction.

In order to assess any differences between patients with high and relatively ‘uncontrolled’ mean arterial pressure (MAP) and normotensives, we measured maternal plasma and cerebrospinal fluid (CSF) UII concentrations along with those in umbilical cord plasma from pre-eclamptic (PET) and normotensive patients undergoing elective Caesarean section under spinal or combined spinal–epidural anaesthesia.

**Materials and methods**

**Patients**

With local regional ethics committee approval and informed written consent, 10 women with pre-eclampsia and 10 with normal pregnancies were enrolled into this single-blinded cohort study. All were to undergo planned Caesarean section under spinal or combined spinal–epidural anaesthesia. Pre-eclampsia was defined according to criteria described by the International Society for the Study of Hypertension in Pregnancy (ISSHP).13 Exclusion criteria were: patient refusal, severe pre-eclampsia (as this may include renal and hepatic failure, which are known to increase UII concentrations14 15), urgent or emergency surgery, general anaesthesia, previous essential hypertension, sedative or opioid analgesic medication within 24 h of surgery, and abnormal renal function.

**Anaesthesia and sampling procedure**

Immediately before spinal or spinal–epidural anaesthesia, an indwelling venous cannula was sited and 5 ml of whole blood collected into EDTA monovette containers containing 3 TTIU of aprotinin, and maintained on ice. These samples were centrifuged at 1500 g for 15 min at 4°C, 1 ml of plasma removed and 1 ml of 1% (v:v) trifluoroacetic acid (TFA) added. During placement of the needle for spinal or combined spinal–epidural anaesthesia, approximately 1 ml CSF was collected and placed into a polypropylene tube and an identical volume of 1% TFA immediately added. Following sample collection anaesthesia and surgery continued. Immediately following delivery of the baby, 5 ml blood was collected from the umbilical vein and processed as for maternal blood. All samples were maintained on ice during preparation and then frozen and stored at −20°C until extraction and assay as a single batch. Patient information, arterial pressures, medication used throughout the pregnancy and details of the infant were taken from the patient’s notes.

**UII extraction and assay**

This was as described previously.16 Samples for extraction (1:1 sample:TFA) were thawed, centrifuged at 12 000 g for 15 min at 4°C and the supernatant loaded (0.3 ml min−1) onto equilibrated (2 ml of 60% acetonitrile in 1% TFA, followed by 3×3 ml washes with 1% TFA at a flow rate of 1 ml min−1) Strata C18-E solid phase extraction cartridges (Phenomenex, Macclesfield, UK). The column was then washed twice with 3 ml of 1% TFA, flow rate approximately 1 ml min−1. Finally, UII was eluted with 2.5 ml of 60% acetonitrile in 1% TFA, and evaporated to dryness in a centrifugal evaporator. Before assay samples were reconstituted in 0.25 ml of radioimmunoassay buffer. We have reported previously 100% recovery and negligible concentrations in plasma-free extracted TFA. Whilst the samples were stored in TFA there was no difference in the median times from collection to extraction in the two groups (208 and 176 days in normotensive and PET groups, no difference, Mann–Whitney) so any peptide loss during storage would not be group-dependent. UII was measured using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, CA, USA) as a single batch according to the manufacturer’s instructions. This assay has a range of 1–128 pg per tube and no cross reactivity with endothelin-1 or angiotensin II. The intra-assay coefficient of variation for this assay was 5.61% based on six mid-range samples (32 pg per tube).

**Data analysis**

Patient characteristics and urotensin data are presented as median (range) with between group comparisons made using Mann–Whitney (unpaired data) or Wilcoxon signed rank tests (paired data) as appropriate. Arterial pressure was measured non-invasively and averages for each day were calculated. A time course for changes in MAP throughout the pregnancy in 4-week epochs from 16 weeks to term was constructed. The data are presented as mean (SD) and between group differences were analysed using general linear model analysis of variance for repeated measures. Statistical tests were performed using SPSS computer software (release 11.01) or GraphPad PRIZM (V3.0). A power calculation based on plasma concentrations of Totsune and
colleagues [mean (sd) plasma UII concentrations of 4.4 (1.0) fmol ml$^{-1}$ in healthy volunteers$^{14}$] suggested that 10 patients per group would detect a 50% difference between control and PET patients ($\alpha=0.05$, $\beta=0.2$).

Results

Patient data

There were no differences between the groups with respect to age or BMI. Systolic, diastolic, and MAPs changed in parallel and for clarity MAP data are presented. There was a significant rise in MAP during the entire course of pregnancy in the PET group ($P=0.003$) and this differed from control from 28 weeks gestation onwards ($P=0.015$ at 28–32 weeks and $P=0.001$ at 32–36 weeks) (Fig. 1). In the PET group, the pregnancy was shorter by approximately 3 weeks and MAP on the day of Caesarean section was elevated. A previous history of PET was more prevalent in the PET group (Table 1). All patients in the PET group were receiving one or more of the antihypertensive medications listed in Table 1. No patients in either group had abnormal renal function; some PET patients had abnormal hepatic function (six with elevated alkaline phosphatase and one with elevated alanine aminotransferase). There were no coagulopathies in either group.

UUI concentrations

Two plasma and two CSF samples were below detection limits in the control and two CSF samples were below detection limits in the PET group. Umbilical cord UII concentrations were slightly higher in the PET group compared with the normotensive group, although this was not statistically significant. Cord UII concentrations were 43% (range 4.9–119%) higher ($P=0.002$, Wilcoxon signed rank) than paired maternal plasma concentrations in PET patients with an elevation seen in all patients. However, overall differences in plasma, CSF and cord UII concentrations between the control and PET groups were not statistically significant (Fig. 2). There was a significant positive correlation between plasma and CSF, plasma and cord and CSF and cord UII concentrations in the PET group (Table 2). Plasma and CSF UII concentrations also correlated in the normotensive group (Table 2). There was a weak but statistically significant negative correlation between gestation (a surrogate for increasing placental age) and cord UII concentrations ($r^2$, $-0.4$, $P=0.049$, $n=10$) in the PET but not the control group.

Discussion

In this study we found no significant difference in plasma, or CSF UII concentrations in patients with pre-eclampsia compared with normotensive controls. Umbilical cord UII concentrations were slightly higher in the PET group but this was not statistically significant. However, umbilical cord UII concentrations were higher than paired maternal plasma

Table 1  Patient details in the control and PET groups presented as mean (range) or number

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($n=10$)</td>
<td>($n=10$)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29 (22–43)</td>
<td>34 (22–40)</td>
</tr>
<tr>
<td>BMI (kg m$^{-2}$)</td>
<td>25 (20–32)</td>
<td>29 (21–46)</td>
</tr>
<tr>
<td>Delivery day MAP (mm Hg)</td>
<td>81 (75–96)</td>
<td>106 (88–128)</td>
</tr>
<tr>
<td>Gestation (days)</td>
<td>274 (267–281)</td>
<td>254 (203–289)</td>
</tr>
<tr>
<td>Previous Caesarean section</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Previous PET</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Smokers</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Medication:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labetol</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Methylxolopra</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Infant sex (boy/girl)</td>
<td>5/5</td>
<td>3/8 (one set of twins)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.24 (2.78–6.13)</td>
<td>1.97 (0.91–3.40)</td>
</tr>
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</table>
Group cord UII levels were higher than paired plasma. There were no differences between the study populations but in the PET group. Overall there was a positive correlation between UII measurements made in the three fluid compartments. There was also a large variation in absolute UII values in both groups.

MAP was significantly higher in the PET group from 28 weeks gestation despite antihypertensive therapy but UII concentrations were not elevated. This information suggests that the rise in MAP is unlikely to be secondary to changes in plasma UII. An alternative explanation may be advanced. In normal pregnancy UT receptor density may be increased in the presence of high circulating UII concentrations making the UT/UII system desensitized. It is therefore possible that in pre-eclampsia responsiveness may be modulated by increasing UT expression. UT expression rather than UII peptide is elevated such that vessels with more receptors become more responsive and hence more constricted resulting in elevated MAP. Based on this, we feel that further studies to determine UT expression differences in these two study groups, with the hypothesis that PET increases UT expression are required.

This is the first study in which umbilical cord UII concentrations have been measured, and in the PET group, these were 40% higher than paired maternal plasma. There are a number of possible explanations for this observation. It is possible that the cord is producing UII but if this were the case then it might be anticipated that a similar elevation would be observed in the normotensive group, unless enhanced production was specific to a PET cord. When plasma from PET patients is incubated with cultured human umbilical vein endothelial cells, increased endothelin-1 secretion was observed. It has been suggested, using an immunocytochemistry technique, that endothelial cells may also produce UII. Our results would be consistent with increased umbilical cord production of this peptide in PET. The effect this would have on cord flow is unclear but in the same study Maguire and colleagues showed a weak constriction of the umbilical vein ex vivo. It would appear from our data that UII may be released, but clearly exerts no effect on maternal arterial pressure above that seen in the normotensive control group, as discussed above. It is also possible that metabolic activity in the cord is reduced in PET, as cord viability decreases, such that apparent UII concentrations are increased.

Several studies have measured UII concentrations in a range of disease states. For example, UII is elevated in renal failure, hepatic cirrhosis with portal hypertension, and diabetes. The situation in heart failure is more variable, with two studies reporting an increase and in one no change was observed. Moreover, in a large study of hypertension clinic patients, plasma UII was elevated by around 50% in the hypertensive group. We reported previously that CSF UII concentrations are low and approximately 15% lower than paired plasma in elderly normotensive and hypertensive males. However, values did not correlate well with arterial pressure, and were not increased in hypertensive relative to normotensive patients, although mean duration of antihypertensive therapy was 4.3 (range 1–8) yr and arterial pressure in the hypertensive group was actually well controlled. In comparison, the present study reports 50% lower plasma and CSF UII concentrations in the normotensive control population, and absolute arterial pressures in the PET group were significantly increased compared with controls. However, the previous study involved elderly (70 yr) males and in this study young (29 yr) pregnant females were recruited. These differences may therefore be attributed to a combination of age and sex. Ng and colleagues have reported lower plasma UII concentrations in women but did not observe any age related differences.

UII regulation of the cardiovascular system is not restricted to peripheral vascular sites but also involves the central nervous system and this was the logic for measurements of CSF concentrations in this and our

**Table 2** Linear regression comparing UII concentrations in maternal plasma, CSF, and cord plasma

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PET</th>
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<tbody>
<tr>
<td>r²</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Plasma vs CSF</td>
<td>+0.59</td>
<td>0.026</td>
</tr>
<tr>
<td>Plasma vs Cord</td>
<td>+0.20</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CSF vs Cord</td>
<td>+0.41</td>
<td>&gt;0.05</td>
</tr>
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</table>

**Fig 2** Plasma, CSF, and umbilical cord plasma (Cord) UII concentrations in normotensive (A) and PET (B) groups. Data from each individual patient are shown and the number of samples is given in parentheses on the x-axis. There were no differences between the study populations but in the PET group cord UII levels were higher than paired plasma.
previous\textsuperscript{16} study. In the absence of human studies some of the clearest data come from work in sheep where intracerebroventricular infusion of UII increased heart rate and cardiac contractility resulting in increased cardiac output and MAP.\textsuperscript{25} Interestingly, peripheral vasodilation was noted, indicating that the increased pressure probably resulted from increased cardiac output.\textsuperscript{25}

In summary, we have failed to detect any differences in plasma, CSF, or umbilical cord UII concentrations in a PET patient population, compared with a normotensive one, despite markedly elevated MAP.

Acknowledgements
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References
1 Bern HA, Lederis K. A reference preparation for the study of bioactive substances in the caudal nervous system of teleosts. \textit{J Endocrinol} 1969; 45(suppl.): xi–xii
3 Douglas SA, Ohlstein HA. Human urotensin-II, the most potent mammalian vasoconstrictor identified to date, as a therapeutic target for the management of cardiovascular disease. \textit{Trends Cardiovasc Med} 2000; 10: 229–37
9 Dogrell SA. Urotensin-II and the cardiovascular system—the importance of developing modulators. \textit{Expert Opin Investig Drugs} 2004; 13: 479–87