Altered endothelial function in isolated human myometrial vessels induced by plasma from women with pre-eclampsia is not reproducible in isolated mouse vessels

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ABSTRACT

In order to facilitate characterization of the circulating factor(s) in pre-eclampsia, the present study aimed to determine whether plasma from women with pre-eclampsia, which induces attenuated endothelial-dependent relaxation in human myometrial arteries, is also capable of inducing altered endothelial function in mouse vessels. Human vessels were isolated from myometrial biopsies taken from women with uncomplicated pregnancies (n = 6). Mesenteric and uterine arteries were isolated from male, female, non-pregnant and pregnant C57B mice (n = 24). Vessels were studied using a wire myograph and incubated with plasma (2%) from women with pre-eclampsia (n = 12) or controls (n = 12). Incubation of myometrial vessels from normal pregnant women with plasma from women with pre-eclampsia reduced endothelial-dependent relaxation. This effect was not reproduced in male or female mouse mesenteric or uterine vessels incubated with plasma from women with pre-eclampsia. In conclusion, there are species-specific differences in the actions of the circulating factor(s) on endothelial-dependent relaxation of human and mouse small arteries.

INTRODUCTION

The multisystem disorder of pre-eclampsia continues to be a leading cause of maternal and perinatal morbidity and mortality. The condition has been the most important cause of maternal death over recent decades and is responsible for the occupancy of approx. 20% of special care baby unit cots [1,2]. Although the aetiology is unclear, there is accumulating evidence for a pathogenic model of pre-eclampsia, whereby a deficiency in the trophoblast invasion of the placental bed spiral arteries leads to a poorly perfused fetoplacental unit. This results in secretion of a factor(s) by the placenta into the maternal circulation which causes ‘activation’ of the vascular endothelium, with the clinical syndrome resulting from widespread changes in endothelial cell function in both small and large vessels [3].

Impaired vascular function in pre-eclampsia has been demonstrated both in vivo [4] and in vitro in a number of vascular beds, including subcutaneous [5], omental [6] and myometrial vessels. Myometrial vessels mounted on a wire myograph, incubated with AVP (arginine vasopressin) and subsequently exposed to incremental doses of the vasodilator agent BK (bradykinin), exhibit significantly attenuated endothelial-dependent relaxation [7].

A number of in vitro studies provide evidence to support the concept of circulating factor(s). Cultured endothelial cells exposed to plasma or serum from women with
pre-eclampsia exhibit a number of altered functional properties [8,9], and plasma from women with pre-eclampsia has been shown to alter microvascular permeability in an animal model [10]. In addition, we have shown that plasma from women with pre-eclampsia, both pre- and post-diagnosis, is capable of altering endothelial-dependent relaxation of myometrial vessels from normal pregnant women [11–13]. Previous preliminary characterization studies have been carried out using myometrial vessels as a bioassay to determine the biological characteristics of the circulating factor(s) responsible for the observed alterations in endothelial-dependent behaviour [14]. Using this technique, the effect of the factor was demonstrated to be reversible, heat-labile, partially removed by charcoal stripping, maintained within a plasma protein concentrate and removed by protease digestion. Extension of such studies to enable characterization of the circulating factor(s) is limited by the availability and biological variability of human myometrial vessels.

Hayman et al. [15] reported that the in vitro effect of plasma from women with pre-eclampsia was specific to myometrial vessels from pregnant women and was not demonstrable in omental arteries or myometrial vessels from non-pregnant women. Gandley et al. [16], and more recently Merchant et al. [17], however, reported that plasma from women with pre-eclampsia was capable of inducing alterations in endothelial function in mouse mesenteric vessels using perfusion myography. Therefore the aim of the present study was to determine whether vessels isolated from C57B mice would serve as a suitable model for extensive characterization studies of the bioactivity of plasma from women with pre-eclampsia.

**MATERIALS AND METHODS**

The Tayside Committee on Medical Research Ethics and Manchester Local Research Ethics Committee gave approval for this work, and written informed consent was obtained for all plasma and tissue samples that were obtained for the study.

**Plasma samples**

Uterine arterial waveform analysis performed at 18–20 weeks gestation is routinely used at Ninewells Hospital, Dundee as a screening test to identify women at high risk of an adverse pregnancy outcome [18]. Following written informed consent, women with either bilateral notches or a normal Doppler waveform analysis at 18–20 weeks gestation were recruited. Patients were recruited prospectively and blood samples were taken at 38 weeks gestation, or at diagnosis, from women who had a normal pregnancy outcome (n = 12) and from women who subsequently developed pre-eclampsia (n = 12). Pre-eclampsia was defined as a blood pressure ≥ 140/90 mmHg on two or more separate occasions after week 20 of pregnancy in a previously normotensive woman in the presence of significant proteinuria (either > 300 mg/l in a 24 h collection or ≥ 2+ on a voided random urine sample in the absence of urinary tract infection [19]).

**Human myometrial samples**

Women with uncomplicated pregnancies at term, who had no known underlying medical disease, fetal abnormality or fetal compromise undergoing planned Caesarean section for indications, such as breech or previous Caesarean section, were recruited. Uterine biopsies were taken from the upper lip of the uterine incision at elective lower segment Caesarean section following delivery of the fetus and placenta.

**Mouse vessels**

C57B male or female adult mice (n = 24) were killed by cervical dislocation following stunning according to national guidelines. Mesenteric or uterine arteries were dissected immediately. Pregnant C57B mice were killed on day 18 (term, 19–20 days).

**Experimental protocol**

**Plasma samples**

Blood was taken using a Vacutainer (Becton Dickinson) and collected in pre-cooled EDTA vials. Samples were then centrifuged for 15 min at 500 g at 4 °C, and the plasma was removed and stored at −80 °C until use. Plasma samples were defrosted on ice and pooled; aliquots of pooled samples were stored at −80 °C and maintained below 4 °C during preparation of experiments.

**Myography**

Myometrial biopsies were dissected in ice-cold PSS [physiological salt solution; 127.76 mmol/l NaCl, 25 mmol/l NaHCO3, 4.69 mmol/l KCl, 2.4 mmol/l MgSO4, 1.6 mmol/l CaCl2, 1.18 mmol/l KH2PO4, 6.05 mmol/l glucose and 0.034 mmol/l EDTA (pH 7.4)] under a stereomicroscope. Myometrial arteries (200–500 μm) were identified, carefully dissected from the surrounding connective tissue and cut into approx. 2 mm sections. Mouse mesenteric or uterine arteries (150–250 μm) were dissected from surrounding connective tissue in ice-cold PSS and cut into 2 mm sections.

Vessels were mounted immediately on a Danish Myotechnology M610 wire myograph. Following completion of the mounting procedure, vessel lengths were measured using a calibrated eyepiece micrometer. Initially, the bath contained 7 ml of PSS, and was gassed with air/5 % CO2. The vessels were then normalized to an internal circumference calculated at 0.9 of L100 (the calculated circumference of the vessel at a passive transmural pressure of 100 mmHg) for the remainder of the experiment.

**Human myometrial arteries**

Plasma was added to the baths at a 2 % final concentration, 1 unit/ml heparin was added to prevent coagulation,
and incubated at 37°C for 1 h [12]. According to our previous experimental protocol [13], vessels were constricted with AVP (arginine vasopressin; 10^{-8} mol/l) and, following a sustained constriction, were exposed to incremental doses of BK (10^{-12}–10^{-6} mol/l). Vessels were then subjected to washes (1–2) with PSS until they returned to their basal tension and the protocol was repeated. For each experiment, a control vessel (incubated with heparin only for the same time period) was run alongside vessels incubated with plasma. Relaxation was expressed as the percentage of tonic constriction.

Comparison of vasoactive agonists

In order to compare the agonist-induced vasoconstriction of mouse and human myometrial arteries, vessels were constricted with incremental doses of AVP (10^{-12}–10^{-8} mol/l) or Phe (phenylephrine; 10^{-9}–10^{-5} mol/l). Magnitude of constriction, at the maximal dose of agonist, and dose–response curves were compared. Following constriction with either Phe or AVP, mouse arteries and human myometrial vessels were subsequently exposed to the endothelial-dependent vasodilators BK (10^{-12}–10^{-6} mol/l) or Ach (acetylcholine; 10^{-9}–10^{-5} mol/l). In order to avoid methodological error, vessels were exposed to a constrictive, followed by a dilatory, agonist using a randomized protocol. The proportion of NO (nitric oxide)/prostacyclin-independent relaxation was assessed by co-incubation with indomethacin (10 µmol/l) and L-NNA (N^{6}-nitro-l-arginine; 100 µmol/l) respectively.

Incubation of mouse arteries with plasma

Plasma was added to the baths at a final concentration of 2%, 1 unit/ml heparin was added to prevent coagulation, and was incubated at 37°C for 2 h. Arteries were constricted with Phe (10^{-5} mol/l) and following sustained constriction exposed to incremental doses of Ach (10^{-9}–10^{-5} mol/l). Relaxation was expressed as a percentage of the tonic constriction.

Statistical analysis

SPSS version 10 was used to analyse the demographic data. Myodata and Graphpad Prism version 3.0 were used to analyse the myography data. All myography data were tested for normality using Graphpad Prism software and are represented as means ± S.E.M. Relaxation curves were compared using repeated-measures ANOVA, and residual constriction at the maximum dose of BK/Ach was compared using Student’s t test. Significance was determined at P < 0.05, and ’n’ reflects the number of patients/animals.

RESULTS

Demographic data for the women from whom plasma samples were obtained

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-eclampsia</th>
<th>Normal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Max SBP (mmHg)</td>
<td>146 (140–182)*</td>
<td>127 (98–134)</td>
</tr>
<tr>
<td>Max DBP (mmHg)</td>
<td>102 (90–110)*</td>
<td>79 (60–90)</td>
</tr>
<tr>
<td>IBR (centile)</td>
<td>3.5 (0–100)*</td>
<td>40 (12–81)</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2225 (750–4750)*</td>
<td>3480 (3000–3945)</td>
</tr>
<tr>
<td>Delivery gestation (weeks)</td>
<td>36 + 1 (27 + 3–40 + 5)*</td>
<td>40 + 6 (38 + 6–41 + 6)</td>
</tr>
</tbody>
</table>

Table 1  Demographic data for patients from whom plasma samples were obtained

Values are medians (range). *P < 0.05 compared with normal outcome. IBR, individualized birth weight ratio.

Figure 1 Effect on endothelial-dependent relaxation of incubating human myometrial arteries for 1 h with plasma taken from women with pre-eclampsia (closed squares) or from women who had a normal pregnancy outcome (open squares)

P < 0.001.

pre-eclampsia had significantly elevated blood pressures (by definition), delivered at an earlier gestation and delivered babies of lower birth weight than women in the normal outcome group.

Human myometrial vessels

Incubation of myometrial vessels from normal pregnant women (n = 6) with pooled plasma from women with pre-eclampsia resulted in a significant attenuation in endothelial-dependent relaxation compared with vessels incubated with plasma from women with uncomplicated pregnancies (residual constriction, 61.4 ± 14.1% compared with 12.1 ± 3.6%; P = 0.02, as determined by Student’s t test; Figure 1).

Response of mouse arteries to vasoconstrictive agonists

Mouse mesenteric and uterine arteries constricted to both AVP and Phe. In mouse arteries from both vascular beds, contractile responses to AVP were oscillatory or phasic, but were sustained to Phe. There was no significant difference between the size of constriction (e.g. active effective pressure at the maximal dose of Phe in mouse mesenteric...
arteries was 16.6 ± 1.9 compared with 22.0 ± 1.4 kPa in human myometrial vessels; \( P = 0.1 \), as determined by Student’s \( t \) test) or sensitivity to Phe (\( P = 0.85 \), as determined by repeated-measures ANOVA) between mouse arteries and human myometrial vessels.

**Endothelial-dependent relaxation**

Mouse mesenteric and uterine arteries pre-constricted with Phe relaxed in a dose-dependent manner to Ach but not to BK (Figure 2A), whereas Phe-stimulated human myometrial vessels relaxed to BK but not to Ach (Figure 2B).

There was, however, no difference in the residual constriction at the maximal dose of Ach in mouse arteries compared with the residual constriction at the maximal dose of BK in human myometrial vessels (residual constriction, 23.1 ± 4.3 compared with 14.1 ± 5.4%; \( P = 0.25 \), as determined by Student’s \( t \) test; Figure 3). Equally, the Ach-dependent NO/prostacyclin-independent relaxation of mouse mesenteric vessels was similar to the BK-dependent NO/prostacyclin-independent relaxation of human myometrial vessels (residual constriction, 56.2 ± 9.6 compared with 55.4 ± 12.7%; \( P = 0.96 \); Figure 3).

**Incubation of mouse arteries with plasma samples**

There was no effect on endothelial-dependent relaxation in Phe-constricted mouse vessels incubated with plasma from women with pre-eclampsia compared with plasma from normal pregnant women. The residual constrictions were 34.4 ± 6.0 compared with 27.1 ± 3.7% in uterine arteries of non-pregnant mice (Figure 4A), 15.5 ± 4.4 compared with 14.6 ± 2.7% in uterine arteries of pregnant mice (Figure 4B), 32.8 ± 5.3 compared with 17.6 ± 5.6% or 30.5 ± 8.0 compared with 24.1 ± 4.3% in mesenteric arteries of male (Figure 4C) or female (non-pregnant) mice (Figure 4D) respectively.

**DISCUSSION**

In a number of *ex vivo* settings, plasma from women with pre-eclampsia has been shown to cause alterations in vascular endothelial cell function. Consistent with our previous studies [11,12], we have demonstrated plasma-induced alterations in human myometrial vessels following incubation with samples taken from women with pre-eclampsia from a different study population. The aim of the present study was to determine whether this effect could be reproduced in isolated mouse vessels in order to facilitate future efforts to characterize the bioactive
effect on endothelial-dependent relaxation of plasma from women with pre-eclampsia on isolated arteries.

Figure 4: Effect on endothelial-dependent relaxation of incubating isolated mouse (A) uterine arteries (non-pregnant), (B) uterine arteries (pregnant), (C) mesenteric arteries (male) and (D) mesenteric arteries (female) for 2 h with plasma taken from women with pre-eclampsia (closed squares) or from women who had a normal pregnancy outcome (open squares). P > 0.05.

Components of plasma responsible for the aberrant vascular function associated with the condition.

Previous studies investigating the effect of plasma from women with pre-eclampsia on endothelial function have used the vasoactive agents AVP and BK. In human myometrial vessels, these agonists have been demonstrated to evoke reproducible vasoconstriction and endothelial-dependent relaxation respectively [12,20,21]. In order to evaluate the effect of plasma on the endothelial function of mouse vessels, the response of these vessels to the same agonists was evaluated. Previous studies that have investigated isolated mouse mesenteric and uterine arteries have used Phe and Ach as the vasoactive agents of choice [22–24]. In the present study, we have shown that, although all vessel types constricted in response to both vasoconstrictive agonists (Phe and AVP), the characteristics of these constrictions were different. Mouse vessels did not exhibit a tonic constriction in response to AVP and, therefore, this agonist was not used in further experiments which compared endothelial-dependent relaxation in mouse arteries. In addition, mouse mesenteric and uterine vessels did not exhibit endothelial-dependent relaxation in response to BK. In contrast, human myometrial vessels did not relax to Ach. These differences were evident over a complete dose–response curve for Ach and BK. This indicates species-specific differences in the receptor-coupled activation of endothelial-dependent relaxation. This could be due to altered endothelial expression of BK or muscarinic receptors and/or a functional diversity of any such receptors between mouse and human vessels. Although mouse mesenteric and human myometrial vessels differed in agonist-mediated endothelial-dependent relaxations, the extent of relaxation was similar, as was the NO/prostacyclin-independent component of agonist-induced vasodilation, attributable to EDHF (endothelium-derived hyperpolarizing factor), in both vascular beds. Thus, although the receptors modulating endothelial-dependent relaxation were different, the magnitude and mechanisms of action were similar, allowing a comparison of the effects of human plasma on mouse and human vessels to be made.

Incubation of isolated mouse small arteries with plasma from women with pre-eclampsia had no effect on endothelial-dependent relaxation compared with vessels incubated with plasma from normotensive pregnant controls. Furthermore, the lack of effect of plasma from women with pre-eclampsia on mice vessels was consistent for all the vascular beds studied; there was no effect of gender, vessel origin or pregnancy. These findings contrast with the previously reported observation that plasma from women with pre-eclampsia is capable of altering endothelial function in isolated mouse arteries [16,17].

There are two possible explanations for this inconsistency. Probable differences in the biological efficacy of the plasma between the three studies may reflect disparate patient characteristics. Given that the aetiology of pre-eclampsia is well known to be broad and poorly defined, in terms of clearly identifiable biological effectors, it is perhaps not surprising that a range of effects may be observed when testing the suitability of a biological assay. Certainly results from our present study, and those of Gandley et al. [16] and Merchant et al. [17], indicate that further experimentation is required before the relaxation of mouse arteries can be used as an indicator of the biological activity of plasma from women with pre-eclampsia. This highlights the need for collaborative studies involving the assessment of plasma collected from patients in different laboratories.

Secondly, in the present study, all vessels were investigated using wire myography, whereas previous studies used perfusion myography; technique differences are therefore another potential contributory factor to the contrasting findings. As the plasma samples used in the present study evoked changes in human myometrial arteries using wire myography and identical experimental conditions, the plasma was not without effect in this system. The primary aim of the present study was to determine whether mouse vessels could be used as a suitable alternative to human vessels in future characterization studies of the circulating factor(s). Although pressure myography is a more physiological method of studying vascular function, wire myography allows a number of vessels to be run in parallel. Characterization studies require large numbers of vessels to test different plasma...
fractions and this precludes the use of pressure myography.

In conclusion, these findings suggest that there may be species-specific differences in the actions of the circulating factor(s) on endothelial-dependent relaxation of human and mouse small arteries. It is therefore unlikely that mouse arteries can be used as a substitute for human vessels in characterization studies.

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