Increased uterine arterial pressure and contractility of perfused swine uterus after treatment with serum from pre-eclamptic women and endothelin-1


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ABSTRACT

The present study was designed to examine the effects of ET-1 (endothelin-1) and serum from PE (pre-eclamptic), HP (healthy pregnant) and HNP (healthy non-pregnant) women on uterine arterial perfusion pressure and uterine contractility. Swine uteri (n = 25) were perfused for a period of up to 11 h, with the aim being to preserve a viable organ. Various concentrations of ET-1 as well as serum from PE, HP and HNP women (n = 10 per group) were administered to the perfused swine uteri and IUP (intrauterine pressure) and IAP (intra-arterial pressure) were recorded. ET-1 produced dose-dependent increases in IUP and IAP. The ET-1 concentration in serum was higher in serum from PE women than in HP and HNP women (P > 0.05). Administration of all serum samples had a contractile effect on the swine uterus, with the greatest effect being seen in HNP women (12.8 ± 5.3 mmHg), followed by PE (9.06 ± 4.2 mmHg) and HP (6.1 ± 4.1 mmHg) women. Statistically significant differences were observed between HNP and PE women (P = 0.048), and PE and HP women (P = 0.021). Increases in IAP following administration of serum from PE women (48.8 ± 20.0 mmHg) were significantly higher (P = 0.024) compared with the effect of serum from HP women (28.4 ± 13.7 mmHg). In conclusion, the findings show that serum from PE women has significant vasoconstrictive and oxytocic effects compared with serum from HP women. In pre-eclampsia, the balance between vasorelaxing and vasoactive substances is disturbed.

INTRODUCTION

Pre-eclampsia, a disorder unique to pregnancy, is one of the leading causes of maternal and fetal mortality and morbidity worldwide [1,2]. Hypertension, proteinuria and oedema are the most common and well-known clinical features in the women affected [3]. The vascular endothelium plays a key role in the regulation of vascular tone, coagulation and blood pressure by synthesizing and releasing potent vasoactive substances such as the vasoconstrictors ET-1 (endothelin-1) and thromboxane A2 and the vasodilators prostacyclin and nitric oxide [4]. It has been hypothesized that endothelial cell dysfunction may be a major pathophysiological mechanism, leading to the cardiovascular complications observed in PE (pre-eclamptic) women [4]. However, the pathway mediating endothelial cell dysfunction is still unknown [5].

To date, only cell culture experiments and in vivo vessel models involving the incubation of serum from PE women or plasma have been carried out in an effort...
to identify the substances involved in these processes [6,7]. In addition, in vitro experiments on vascular and uterine reactivity, with isolated arterial rings from various arteries, or uterine strips or rings, have been performed using only the vasoactive components, rather than serum from PE women [8]. As a primary site of pre-eclampsia, the uterus itself has never been examined as a complete organ with intact anatomical structures in relation to this question.

The present study is the first in which the effect of serum from PE women has been examined in a perfused animal uterus. Perfusion models of various organs have been of great interest, particularly in the field of transplantation medicine and in studies of the physiology and metabolism of tissues. We have demonstrated previously [9] that the perfusion model used in the present study is capable of keeping the swine uterus in a functional condition for up to at least 7 h and that it is appropriate for the study of physiological problems. The experimental system detects electrical and mechanical activities in the whole organ, as it maintains the architecture and intercellular relations of the uterus [10,11]. The present study examined the effect of ET-1, the most potent vasoconstricting substance in humans, on IUP (intrauterine pressure) and IAP (intra-arterial pressure) [12], and the effect of serum from HNP (healthy non-pregnant) women, PE women and HP (healthy pregnant) women on IUP and IAP. The aim was to test the hypothesis that serum from PE women contains more vasoconstrictive mediators than serum from HP women. In addition, the possible contractile effect of serum from PE women on the uterus was examined, since it is known that many vasoconstrictors induce myometrial contractions [13] and that many women with preterm births have similar implantation abnormalities to those seen in PE women [14,15].

**METHODS**

**Swine uteri**
Swine (*Sus scrofa domestica*) are widely used in research. The female reproductive system in swine has a bicornate uterus with tortuous fallopian tubes. The fallopian tubes in an adult female have the same diameter as those in humans, but they are much longer. The sow has an oestrous cycle of 20–21 days.

Swine uteri (*n* = 25) were obtained from the local slaughterhouse. They were selected on the basis of their size, overall condition and the condition of the uterine arterial stumps. The mean weight of the swine uteri was 117.27 g (range, 68.5–185.8 g), and all came from healthy animals aged 5 months to 1.5 years. On the basis of previous observations involving perfusion experiments [9], it was decided that the ideal size of uteri for the experiments would be approx. 90–100 g. The swine uteri were very easily separated from the rest of the body in approx. 2 minutes shortly after the animal had been killed by electric shock (1.5 A, 400 V, 4 s).

**Perfusion system**

After cannulation of both uterine arteries with 16–24-gauge needles (Abbocath-T, Abbott, Ireland), depending on the size of the uterus, the organ was placed in a controlled-temperature perfusion chamber (Karl Lettenbauer, Erlangen, Germany) filled with perfusion medium [Krebs-Ringer bicarbonate/glucose buffer (pH 7.4); Sigma, Deisenhofen, Germany]. The uterus was then connected bilaterally to two reservoirs containing perfusion medium.

The perfusion medium was oxygenated with carbogen gas (a mixture of 95 % O₂ and 5 % CO₂) and then pumped into the uterine arterial catheters with two roller pumps. The flow rates of the perfusion medium and oxygenation were constantly monitored and kept at 15 ml/min and 0.05 bars respectively, with an ideal pressure rate of 100 mmHg being maintained throughout the duration of the experiments. ET-1 was purchased from Alexis Biochemicals (San Diego, CA, U.S.A.) and oxytocin (Syntocinon) from Novartis (Nuremberg, Germany).

In the first experiment, ET-1 was administered as a bolus dose (diluted in 1 ml of perfusion medium) in a random order of concentrations (for IUP, 10–2000 ng/ml; for IAP, 5–400 pg/ml). A 30 min pause was observed before each administration to prevent saturation effects. In the second experiment, serum samples (1 ml) were administered at intervals of 30 min. The contractility of the perfused uterus was checked between serum administrations by administering a bolus of oxytocin (0.5 international unit/ml) for IUP measurements or ET-1 (5 pg/ml) for uterine arterial pressure measurements. The uterus was used for further experiments only when the pressure increases after the application of these two control substances were within an acceptable range (oxytocin: median, 7.55 mmHg; maximum, 8.3 mmHg; minimum, 6.0 mmHg; ET-1: median, 14.3 mmHg; maximum, 15.5 mmHg; minimum, 12.9 mmHg) (Figure 1).

**Vitality parameters**

Samples of the perfusate were taken at 1 h intervals after collecting the medium for measurements of pH, Po₂ (partial O₂ pressure), PCO₂ (partial CO₂ pressure), carbonate, lactate and oxygen saturation (for details, see [9]).

**IUP and IAP measurement**

IUP was recorded with an intrauterine balloon catheter (catheter set for use with hysterosalpingo-contrast sonography; Rüschi, Kernen, Germany). The catheter was fixed in the uterine cavity by inflating the balloon with approx. 1 ml of air. The intrauterine catheter was then connected to a pressure sensor (Elwo, Munich, Germany) to convert the IUP changes into electrical signals. The
electrical signals were then transmitted to an analogue-digital transformer (Metex, Munich, Germany) and recorded on a PC. IAP was recorded directly by connecting the arterial cannula to the Urubar DL8-SF electronic semiconductor pressure sensor and the MPR 1 Data-logger (Raumedic, Erlangen, Germany).

Study patients

PE women \((n=10)\) and normotensive HP women \((n=10)\) were recruited from the perinatal and labour ward at the University of Erlangen-Nuremberg and included in the study. Ten HNP women \((n=10)\) from the hospital staff were asked to volunteer (Table 1). All of the study subjects were of Caucasian ethnicity. Only women with singleton pregnancies and with no history of diabetes mellitus, angiopathy or autoimmune disease were included in the study. The study was approved by the Ethics Committee at the University of Erlangen-Nuremberg, and informed written consent was obtained from all of the women included. Blood was collected on admission to the labour and delivery ward, and before the administration of any medication, centrifuged and the serum was stored at \(-40^\circ C\). Pre-eclampsia was assessed on the basis of increased blood pressure (\(>140/90\) mmHg) occurring in a pregnant woman after 20 weeks’ amenorrhoea, accompanied by proteinuria (\(>0.3\) g/24 h) as defined by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [3]. HP women were considered to be normotensive if their blood pressure was \(<140/90\) mmHg. None of the control patients had proteinuria.

Estimation of ET-1 concentrations

A quantitative two-step chemiluminescent sandwich ELISA (ET-1; R & D Systems, Freiburg, Germany) was used for quantitative assessment of ET-1.

Statistical analysis

A paired Student \(t\) test was used for statistical evaluation of significant differences between two groups. \(P < 0.05\) was considered statistically significant. Pressure differences between HNP and PE, as well as PE and HP, groups were evaluated using the exact Wilcoxon rank sum test with Bonferroni-adjusted \(P\) values in order to adjust for the multiplicity of tests. In addition, the effect sizes were inspected using 97.5% CI (confidence intervals) for the difference in medians. \(P < 0.05\) was considered significant. Statistical analyses were performed using SPSS, version 11, and the R system for statistical computing (R Core Development Team, 2004, version 2.0.1).

RESULTS

Concentrations of ET-1 in serum

ET-1 concentrations were higher in serum from PE women \((1.30 \pm 0.87\) pg/ml) than in serum from HP women \((0.72 \pm 0.32\) pg/ml) and HNP women \((0.468 \pm 0.24\) pg/ml), although the difference did not reach statistical significance.

Effect of ET-1 on IUP and IAP

ET-1 produced IAP increases of up to 250 mmHg (Figures 2, 3c and 3d). The effect was dose-dependent. The effect in arterial pressure was immediate; at small concentrations (\(<50\) pg/ml) it lasted for less than 1 min and at higher concentrations (\(>100\) pg/ml) it lasted for 2–8 min. Increases in IUP occurred after the administration of at least 400 pg/ml ET-1, with a maximum at 1 \(\mu\)g/ml ET-1, when a plateau was reached. These high amounts of ET-1 induced several rhythmic uterine contractions.

Effect of serum bolus administration

Each administration of serum (1 ml) led to pressure rises in both the uterus and uterine arteries (Figures 3a, 3b and 4).

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Figure 2 Increases in uterine arterial perfusion pressure and IUP after the administration of ET-1

As shown in Figure 4 (upper panel), serum from HNP women showed the greatest effect in increasing IUP (12.8 ± 5.3 mmHg), followed by serum from PE women (9.06 ± 4.2 mmHg). The lowest pressure increases were induced by serum from HP women (6.1 ± 4.1 mmHg). Statistically significant differences were observed between HNP and PE women [P = 0.048, with estimated difference of 3.1 mmHg (CI, 0.0–7.1 mmHg)] and PE and HP women [P = 0.021, with estimated difference of 3.15 mmHg (CI, 0.5–5.0)].

IUP pressure

As shown in Figure 4 (lower panel), serum from PE women caused the greatest increases in perfusion pressure (48.8 ± 20.0 mmHg), followed by serum from HNP women (46.6 ± 15.4 mmHg). The lowest pressure increases were caused by serum from HP women (28.4 ± 13.7 mmHg). Increases in IAP following administration of serum from PE women were significantly higher in comparison with the effect of serum from HP women [P = 0.02, with estimated difference of 20 mmHg (CI, 5–36)].

DISCUSSION

Pre-eclampsia is the most common disease specific to pregnancy, causing fetal growth restriction, and is therefore a major cause of perinatal mortality and...
Perfused pig uterus as model for pre-eclampsia

Figure 4 Increases in IUP (upper panel) and IAP (lower panel) after introduction into the uterine perfusion system of serum (1 ml bolus) from HNP women, PE women and HP women.

Bars indicate means. n = 10 in each group.

morbidity. It complicates 6–8% of all pregnancies longer than 20 weeks, but the aetiology is as yet unknown [2,3,5]. One theory for the pathophysiology involves a hyperlipidaemic genesis, leading to high lipid concentrations and an inappropriate antioxidative response to oxidative processes and endothelial damage. Another theory suggests an inadequate immunological tolerance for the fetus, leading to cytokine release. Some studies have suggested a genetic defect in endothelial NOS (nitric oxide synthase) in the renin-angiotensin system. Mitochondrial defects might also explain the incomplete invasion of cytotrophoblasts into the maternal endometrium (reviewed in [5]).

The proposed model is that a reduced blood supply to the placenta results in the production of unknown factors, which are released into the maternal circulation and act on endothelial cells, leading to endothelial dysfunction [16–18]. Several endothelial markers have been found to be elevated in pre-eclampsia, such as cellular fibronectin, von Willebrand factor, tissue plasminogen activator and plasminogen activator inhibitor [19,20]. In addition, enhanced endothelial expression of adhesion molecules has been reported in pre-eclampsia [21].

Pre-eclampsia is also associated with increased levels of plasma ET-1 [22–24], and a causative role for ET-1 in pre-eclampsia has been suggested by the observation that raised levels of plasma ET-1 in pregnant sheep, produced by continuous systemic infusion of ET-1, resulted in cardiovascular and haemodynamic changes that in many ways resemble the human disease of pre-eclampsia [25].

The increased levels of ET-1 in PE women rapidly return to the normal postnatal levels of HNP women within 48 h of delivery, as might be expected on the basis of the prompt clinical resolution of this disorder [26]. These data suggest that ET-1 may be involved in the mechanisms of vasospasm associated with pre-eclampsia [22].

The best-known action of ET-1 is as a vasoconstrictor. Injection of a single dose of ET-1 produces an initial decrease in systemic blood pressure, followed by a prolonged increase in blood pressure lasting for 1–3 h [27]. In addition to their actions as vasoconstrictors, endothelins also produce a variety of other biological effects. These include stimulation of cardiac contraction, regulation of the release of vasoactive substances and stimulation of smooth muscle mitogenesis. Endothelins also stimulate contraction of most smooth muscles and stimulate secretion by tissues such as kidney, liver and adrenal glands [28].

Many studies have hypothesized that serum or plasma in PE women may contain increased concentrations of vasoconstrictive substances compared with HP women [16,18,29]. The aim of the present study was to verify this hypothesis. In addition, the intention was to test the intrauterine contractile effect of serum, since it is known that vasoactive substances such as prostaglandins are also oxytocic [9], and one-third of women with preterm births have implantation abnormalities identical with those in PE women [14,15].

Vasoactive drugs have been investigated by various research groups in human and animal organ perfusion models in recent decades [30,31]. In the present study, a swine uterus perfusion model was developed in order to overcome the problems arising from the small numbers and the poor condition of the human uteri that can be obtained for experimental purposes [9].

Swine have been increasingly used as biomedical research models over the last 20 years, as they are recognized as a suitable animal model for human disease on the basis of comparable anatomy and physiology [30,32]. In order to avoid in vivo animal experiments, it was decided to use uteri from freshly killed animals from the slaughterhouse. Logistically, this had the advantage that obtaining a large number of uteri every day only took a matter of minutes and, in addition, approval from an Ethics Committee or Animal Experimentation Board was not needed, as no animals were killed for experimental purposes. Moreover, the uteri were derived from healthy young animals in their reproductive years.

Normally, the uterus would rapidly degenerate without constant perfusion. A complex perfusion system was therefore established that provided the uteri with a steady flow rate of circulation, a constant temperature and continuous oxygenation of the perfusion

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medium, simulating physiological conditions in every way possible.

Using this perfusion model, it was possible to demonstrate that serum from all of the women included in the study had a vasoconstrictive effect. The most potent effect was observed in serum from PE women, followed by serum from HNP and HP women. The difference in the vasoconstrictive effect of serum from HP women and that from the PE women was statistically significant, whereas the difference in the perfusion pressure increase between serum from HNP women and that from PE women did not reach significance. This might be explained by the presence of either increased circulating concentrations of vasodilators or decreased concentrations of contractile factors in healthy pregnancy, whereas this balance is massively disturbed in PE women. These findings are in accordance with those of Neal et al. [33], who demonstrated in an in vivo animal experimental model that plasma from women with mild pre-eclampsia and normotensive women did not increase vascular permeability, whereas plasma from women with severe pre-eclampsia induced a statistically significant increase in vascular permeability.

Serum from HP women produced intra-uterine contractions with lower amplitude compared with serum from PE women, suggesting that serum from HP women contains mediators that have a tocolytic effect.

The effects of ET-1 on IAP and IUP were investigated in the present study. The results were dose-dependent and statistically significant. ET-1 was the only substance tested in the present set of experiments, as it is the most potent vasoconstrictive substance [12], and it was used as an example of the potential of this experimental swine uterus perfusion system.

Serum concentrations of ET-1 were also investigated in the group of patients studied. ET-1 concentrations were higher in PE women, followed by HP women and finally by HNP women, although the differences were not significant. Since the difference in ET-1 concentrations measured in serum between PE and HP women was too low to cause the observed perfusion pressure rise difference, ET-1 is unlikely to be the only factor responsible for pressure increase. The identification of these additional factors and the importance of the protein binding of ET-1 remains a topic for further studies. This animal perfusion system may be helpful for this purpose.

Conclusions
In a novel set of experiments, the intra-arterial and intra-uterine effects of serum from PE women, HNP women and HP women on an isolated swine uterus were examined. The findings show that serum from PE women has significant vasoconstrictive and oxytocic effects compared with serum from HP women. ET-1 also showed vasoconstrictive and oxytocic activity in the swine uterus perfusion system. These experiments demonstrate that there must also be other mediators of the vascular changes observed in the disease that require further examination. The swine perfusion model is an appropriate experimental model for the study of vascular reactivity and uterine contractility, especially in disorders such as pre-eclampsia.

ACKNOWLEDGMENTS
T. M. was supported by the German Academic Exchange Service (DAAD). This work was funded by institutional sources. We would like to thank Dr W. Wolf from the local slaughterhouse (Department of Animal Health, Stadt Erlangen, D-91054 Erlangen, Germany) for his support and donation of swine uteri.

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Received 29 November 2004/18 April 2005; accepted 28 April 2005
Published as Immediate Publication 28 April 2005, DOI 10.1042/CS20040340