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Abnormal uterine artery remodelling in the stroke prone spontaneously hypertensive rat

Heather Y. Small*, Hannah Morgan, Elisabeth Beattie, Sinead Griffin, Marie Indahl, Christian Delles, Delyth Graham

Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

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ABSTRACT

Introduction: The stroke prone spontaneously hypertensive rat (SHRSP) is an established model of human cardiovascular risk. We sought to characterise the uteroplacental vascular response to pregnancy in this model and determine whether this is affected by the pre-existing maternal hypertension.

Methods: Doppler ultrasound and myography were utilised to assess uterine artery functional and structural changes pre-pregnancy and at gestational day 18 in SHRSP (untreated and nifedipine treated) and in the normotensive Wistar-Kyoto (WKY) rat. Maternal adaptations to pregnancy were also assessed along with histology and expression of genes involved in oxidative stress in the placenta.

Results: SHRSP uterine arteries had a pulsatile blood flow and were significantly smaller ($70906 \pm 3903\ \mu m^2$ vs. $95656 \pm 8524\ \mu m^2$ cross-sectional area; $p < 0.01$), had a significant increase in contractile response (57.3 ± 10.5 kPa vs 27.7 ± 1.9 kPa; $p < 0.01$) and exhibited impaired endothelium-dependent vasorelaxation ($58.0 \pm 5.9\%$ vs $13.9 \pm 4.6\%$; $p < 0.01$) compared to WKY. Despite significant blood pressure lowering, nifedipine did not improve uterine artery remodelling, function or blood flow in SHRSP. Maternal plasma sFLT-1/PlGF ratio (5.3 ± 0.3 vs 4.6 ± 0.1; $p < 0.01$) and the urinary albumin/creatinine ratio (1.9 ± 0.2 vs 0.6 ± 0.1; $p < 0.01$) was increased in SHRSP vs WKY. The SHRSP placenta had a significant reduction in glycogen cell content and an increase in *Hif1α*, *Sod1* and *Vegf*.

Discussion: We conclude that the SHRSP exhibits a number of promising characteristics as a model of spontaneous deficient uteroplacental remodelling that adversely affect pregnancy outcome, independent of pre-existing hypertension.

1. Introduction

In human pregnancy, the cardiovascular (CV) system undergoes major adaptations in response to the developing fetus. A central change in the CV system is a steady increase of 40–50% in blood volume [1]. The resulting increase in cardiac output is accommodated by angiogenesis [2] and, predominantly, the adaptation of existing vasculature in order to stabilise blood pressure by reducing total peripheral resistance [3]. In particular, the remodelling of the uteroplacental vasculature plays a critical role in sustaining an adequate blood flow at the maternal–placental interface, thus providing the developing fetus with appropriate nutrients and oxygen. Deficiencies of the maternal uterine vasculature have been shown to be present in women with gestational hypertension [4], pre-eclampsia [5], fetal growth restriction [6] and recurrent pregnancy loss [7]. These conditions significantly contribute to maternal and fetal morbidity and mortality worldwide. Despite the impact of these pregnancy-related complications, there is a lack of understanding in the underlying pathophysiological mechanism of how and why the uteroplacental vasculature does not remodel sufficiently. The ability to study these underlying mechanisms of abnormal remodelling in human uteroplacental vasculature during pregnancy is limited and the use of small animal models has contributed greatly to this field. In particular, the rat shows a similar maternal vascular response to pregnancy [8] and exhibits the same haemochorial placental type as humans [9]. However, currently, there are no models which exhibit spontaneous deficient uterine artery remodelling accompanied by reduced uteroplacental blood flow without pharmacological, transgenic or surgical

* Corresponding author. Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, 126 University Place, Glasgow G12 8TA, UK.
E-mail address: h.small.1@research.gla.ac.uk (H.Y. Small).

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intervention.

The incidence of cardiovascular disease (CVD) in women of child-bearing age is increasing [10]. Despite a similar percentage of pregnancies being affected by pre-existing CVD (3–5%) [10–12] as some forms of pregnancy-specific hypertension such as pre-eclampsia; it has received relatively less research attention. Deficiencies in pregnancy-related vascular remodelling may be related to pre-existing CV risk as the adaptation of the CV system can be likened to a stress test causing these risk factors to be manifested clinically as pregnancy induced hypertension [13]. The stroke prone spontaneously hypertensive rat (SHRSP) is an established model of genetic predisposition to CVD and mimics many of the human traits observed in CVD. These rats are characterised by spontaneous hypertension and a higher rate of cardiovascular complications compared to the control strain; the Wistar Kyoto (WKY) rat [14]. Previous studies have briefly characterised pregnancy in the SHRSP [15] and have indicated that placental amino acid transport is deficient in these animals [16] however the response of the uterine vasculature to pregnancy in the SHRSP has not been studied. We hypothesized that pre-existing maternal CV risk in SHRSP would result in deficient remodelling of the uteroplacental vasculature in response to pregnancy. We aimed to characterise how SHRSP and WKY responded to pregnancy by analysing the structure and function of the uterine arteries before and during pregnancy as well as characterising the effects this may have on maternal and placental adaptation and fetal outcome. Furthermore, we carried out an intervention study in SHRSP only, using nifedipine to distinguish whether deficiencies in uterine vascular remodelling present in this model were dependent upon the presence of pre-existing hypertension.

2. Materials and methods

2.1. Animals

Female SHRSP and WKY were recruited to the study at 7 weeks of age. All animals were housed under controlled lighting (from 0700 to 1900 h) and temperature (21 ± 3 °C) and received a normal diet (rat and mouse No.1 maintenance diet, Special Diet Services). All animal procedures were approved by the Home Office according to the Animals (Scientific Procedures) Act 1886 (Project License 60/4286). All females were age-matched and time mated at 12 weeks of age (±4 days). Virgin animals were used at 15 weeks of age. Day 0 of pregnancy was defined as the day that a coital plug was observed indicative of successful mating having taken place.

For vascular studies, animals were split into two sub-groups where one was used to measure blood pressure by radiotelemetry and allowed to progress to parturition (untreated WKY, untreated SHRSP and nifedipine treated SHRSP; n = 6 in each group) and the other was used for ultrasound and sacrificed at gestational day (GD) 18 for *ex vivo* pressure and wire myography (untreated WKY, untreated SHRSP and nifedipine treated SHRSP; n = 6 in each group). For biochemical measurements and placental histology, untreated pregnant SHRSP and WKY rats (n = 8 in each group) were sacrificed at GD 18. For additional fetal and placental measurements untreated SHRSP (n = 4) and WKY rats (n = 4) were harvested at GD 14 and 20.

2.2. Nifedipine treatment of SHRSP

7 week old SHRSP began nifedipine treatment at 25 mg/kg/day administered in two doses: a 10 mg/kg/day dose mixed in a 1 ml aliquot of baby food and a 15 mg/kg/day dose in drinking water in order to maintain lowered blood pressure throughout the 24 h period. Stock solutions of nifedipine in drinking water were prepared in ethanol and diluted to the appropriate concentration with no more than a 0.8% final ethanol concentration.

2.3. Blood pressure measurement

Blood pressure from 7 to 10 weeks was measured using tail cuff plethysmography [17]. A radiotelemetry transmitter (calibration accurate within ± 6 mmHg) was implanted at 10 weeks into untreated WKY, untreated SHRSP and nifedipine treated SHRSP (n = 6) as previously described [18]. Systolic and diastolic blood pressure, heart rate and activity were then monitored using the Dataquest system (Data Sciences International).

2.4. Ultrasound and Doppler analysis of the uterine arteries

Uterine artery Doppler waveform recordings were used to assess *in vivo* uteroplacental blood flow in untreated SHRSP, nifedipine treated SHRSP and untreated WKY rats. Rats were lightly anaesthetized throughout the procedure at approximately 1.5% isoflurane in oxygen. Rats were imaged trans-abdominally using an Acuson Sequoia c256 ultrasound imager fitted with a 15-MHz linear array transducer. Peak systolic velocity (PSV) and end diastolic velocity (EDV) was measured from 6 consecutive cardiac cycles. Resistance index (RI) (RI = [PSV−EDV]/PSV) and S/D ratio (PSV/EDV) were calculated.

2.5. Wire and pressure myography of the uterine arteries

Virgin and pregnant animals were age matched at the point of sacrifice (GD 18). Uterine arteries were harvested from the same area of the horn (i.e. closer to the vagina than the ovary), and which contained the most fetuses. Arteries were not harvested from a horn that had less than four fetuses. Arteries were dissected in calcium free physiological salt solution (PSS) solution. Sections of uterine artery (4.8–5.2 μm in length) were mounted on the wire myograph (AD Instruments), normalized and subject to a wake up procedure as previously described [19]. To establish the vessel's contractile response, noradrenaline was added at the following increasing concentrations: 1×10^{-9}, 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7}, 3×10^{-7}, 1×10^{-6}, 3×10^{-6}, 1×10^{-5} and 3×10^{-5} M. To determine the vessel's endothelial dependent relaxation response, vessels were pre-constricted with 3×10^{-5} M noradrenaline followed by the addition of carbachol at the following increasing concentrations: 1×10^{-8}, 3×10^{-8}, 1×10^{-7}, 3×10^{-7}, 1×10^{-6}, 3×10^{-6} and 1×10^{-5} M. The pressure myograph system (Danish Myo Technology) was set up and equilibrated according to manufacturer's instructions. Vessels were maintained at 37 °C and 95% O_2 and 5% CO_2 throughout the experiment. Vessels were subject to a pressure curve at: 10, 20, 40, 60, 80, 100 and 110 mmHg. Measurements of internal (D_i) and external diameter (D_e) were taken after 5 min at each pressure. Wall thickness was calculated as $[(D_e - D_i)/2]$. Cross sectional area was calculated as $[(\pi/4) \times (D_e^2 - D_i^2)]$. Wall strain was calculated as $[D_i/D_e]$. Wall stress was calculated as [(133.4 × pressure mmHg × D_i)/2 × wall thickness] where 1 mmHg = 133.4 dyn/cm^2. Wall stress was then divided by 10^6 to give x10^6 dyn per cm^2.

2.6. Maternal, placental and fetal characterisation

Water intake and urine output pre-pregnancy and at different gestational time points were monitored by housing the animals in a metabolic cage for 24 h. Animals were acclimatised to the metabolic cage prior to use. Urine and extracted plasma were stored at −80 °C until use. Animals were sacrificed at GD 18 when blood was collected by cardiac puncture and fetal and placental tissue

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