S-Nitrosoglutathione improves haemodynamics in early-onset pre-eclampsia

Thomas R. Everett,1 Ian B. Wilkinson,2 Amita A. Mahendru,1 Carmel M. McEniery,2 Stephen F. Garner,3,4 Alison H. Goodall5 & Christoph C. Lees6,7

1Fetal Medicine Department, Rosie Hospital, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 2QQ, UK, 2Clinical Pharmacology Unit, University of Cambridge, Cambridge CB2 0QQ, UK, 3Department of Haematology, University of Cambridge, Cambridge CB2 0PT, UK, 4National Health Service Blood and Transplant, Cambridge CB2 0PT, UK, 5Department of Cardiovascular Sciences, University of Leicester and NIHR Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester LE3 9QP, UK, 6Centre for Fetal Care, Queen Charlotte’s and Chelsea Hospital, Imperial Healthcare NHS Trust, London W12 0HS, UK and 7Department of Development and Regeneration, University Hospitals Leuven, Leuven B-3000, Belgium

AIMS
To determine the effects of in vivo S-nitrosoglutathione (GSNO) infusion on cardiovascular function, platelet function, proteinuria and biomarker parameters in early-onset pre-eclampsia.

METHODS
We performed an open-label dose-ranging study of GSNO in early-onset pre-eclampsia. Six women underwent GSNO infusion whilst receiving standard therapy. The dose of GSNO was increased incrementally to 100 μg min⁻¹ whilst maintaining blood pressure of >140/80 mmHg. Aortic augmentation index, aortic pulse wave velocity, blood pressure and maternal–fetal Doppler parameters were measured at each dose. Platelet P-selectin, protein-to-creatinine ratio and soluble anti-angiogenic factors were measured pre- and postinfusion.

RESULTS
Augmentation index fell at 30 μg min⁻¹ S-nitrosoglutathione (−6%, 95% confidence interval 0.6 to 13%), a dose that did not affect blood pressure. Platelet P-selectin expression was reduced [mean (interquartile range), 6.3 (4.9–7.6) vs 4.1 (3.1–5.7)% positive, P = 0.03]. Soluble endoglin levels showed borderline reduction (P = 0.06). There was a borderline significant change in pre-to-postinfusion protein-to-creatinine ratio [mean (interquartile range), 0.37 (0.09–0.82) vs. 0.23 (0.07–0.49) g mmol⁻¹, P = 0.06]. Maternal uterine and fetal Doppler pulsatility indices were unchanged.

CONCLUSIONS
In early-onset pre-eclampsia, GSNO reduces augmentation index, a biomarker of small vessel tone and pulse wave reflection, prior to affecting blood pressure. Proteinuria and platelet activation are improved at doses that affect blood pressure minimally. These effects of GSNO may be of therapeutic potential in pre-eclampsia, a condition for which no specific treatment exists. Clinical studies of GSNO in early-onset pre-eclampsia will determine whether these findings translate to improvement in maternal and/or fetal outcome.
Introduction

Pre-eclampsia is a multisystem disorder, which manifests clinically as hypertension and proteinuria. Pre-eclampsia occurring at or close to term is usually treatable by delivery, with minimal risk to mother or baby. However, the condition is commonly more severe when the onset is early, particularly before 32 weeks of gestation, which affects ~1% of pregnancies. In this situation, expeditious conservative management, focusing on control of hypertension and seizure prevention, to gain fetal maturity is key, but there is no current treatment that targets the underlying pathophysiology [1, 2].

The underlying pathological processes of pre-eclampsia are hypothesized to occur in two stages [3]. Abnormal placentation is suggested to be the initiating event, resulting in reduced placental perfusion, which in turn leads to increased oxidative stress, which, in combination with a maternal predisposition, results in endothelial dysfunction. This is manifest by changes in a number of signalling pathways and homeostatic mechanisms, but impaired nitric oxide (NO) bioavailability [4] is thought to play a major role in the maternal manifestations of pre-eclampsia, such as hypertension, platelet activation, proteinuria and oedema.

Decreased bioavailability of NO provides a potential therapeutic target for novel drug therapy of pre-eclampsia. Nitric oxide donors, used in a research context, reduce blood pressure (BP) and platelet activation in pre-eclampsia whilst having no detrimental effect on placental perfusion [1, 5]. Prophylaxis with glyceryl trinitrate in high-risk women reduces overall adverse outcome, though not the incidence of pre-eclampsia [2, 6]. However, the utility of most NO donors, including glyceryl trinitrate, is limited by hypotension, side-effects and tachyphylaxis. S-Nitrosoglutathione (GSNO) causes less hypotension at doses that affect platelet aggregation [3, 7], has minimal reported side-effects [4, 8] and does not cause tachyphylaxis [9].

We sought to investigate the physiological effects of GSNO in early-onset pre-eclampsia, using augmentation index (AIx) as a surrogate measure for NO bioavailability. Augmentation index is a measure of wave reflection and thus provides information about the tone of small arteries and arterioles, which has previously been shown to be highly dependent on nitric oxide. Accordingly, we performed an open-label dose-ranging study to determine whether potentially beneficial effects, particularly reduction in pulse wave reflection, occur whilst maintaining a safe BP, and without causing evidence of acute fetal compromise.

Methods

Participants

Women with a singleton pregnancy between 24+0 and 32+0 weeks of gestation presenting to the Rosie Maternity Unit, Addenbrooke’s Hospital (Cambridge, UK) between August 2010 and April 2012, with pre-eclampsia as defined by the International Society for the Study of Hypertension in Pregnancy [10], were approached to participate in the study. All had a systolic BP >160 mmHg or diastolic BP >100 mmHg prior to GSNO infusion. Women aged <18 years, those with multiple pregnancies, fetal venous Doppler abnormalities or short-term variation <4.5 ms on computerized cardioto- cography, or those deemed to require immediate delivery by the responsible clinical team were not approached. Ethical approval was granted by the local research ethics committee (08/H0304/46), and the investigators had no participation in the ongoing clinical care of women during, or following, the GSNO infusion. The study conforms to principles outlined in the Declaration of Helsinki, and informed written consent was obtained from all participants.

Ultrasound

Transabdominal Doppler measurements were performed using Samsung Medison Accuvix XG (Seoul, South Korea) ultrasound equipment and a 3DC2-6 transabdominal probe. Uterine artery waveforms were obtained as previously described [11], and the mean pulsatility index (PI) value was recorded. Fetal umbilical, middle cerebral and ductus venosus Doppler waveforms were obtained according to standard methodology [12].

Aortic pulse wave velocity

Aortic pulse wave velocity (aPWV) was assessed between the carotid and femoral pulse sites using a Vicorder device (Skidmore Medical, Bristol, UK), as described elsewhere [13]. To avoid the gravid uterus potentially overestimating the horizontal path, the suprasternal notch–femoral distance was measured using callipers.

Augmentation index

Pulse wave analysis was performed at the radial artery by applanation tonometry using a micromanometer (Millar Instruments, Houston, TX, USA) and SphygmoCor device (AtCor Medical, West Ryde, NSW, Australia) as previously described [14]. The aortic pulse waveform was derived from the radial artery waveform using a validated transfer function. The Alx adjusted for a standard heart rate of 75 beats min⁻¹, Alx-75, is calculated automatically by the SphygmoCor device. The Alx is calculated as augmentation pressure/pulse pressure × 100 and expressed as a percentage. Heart rate was also recorded from the SphygmoCor device.

Blood pressure

Brachial BP, using the left arm, was obtained using an Omron M7 automated sphygmomanometer, a device validated for use in pregnancy [15].
Measurements
All measurements were taken 30 min and immediately prior to infusion. Subsequent to this, AIx, aPWV and ultrasound measurements were taken every 30 min until 1 h following cessation of the infusion. Blood pressure and HR were measured every 5 min for 15 min following a change of infusion rate and then every 15 min if BP remained stable or until further change in infusion rate.

S-Nitrosoglutathione
S-Nitrosoglutathione was custom produced to good manufacturing practice (GMP) standard by Onyx Scientific (Sunderland, UK) and stored in darkened vials in 100 μg aliquots at −40°C until immediately prior to infusion. Prior to infusion, GSNO was diluted to the desired concentration using 0.9% normal saline and stored, protected from light, at 4°C.

The infusion was performed through an 18 gauge intravenous cannula in the antecubital fossa. Following baseline measurements, GSNO infusion was commenced at 1 μg min⁻¹ and increased to 3, 10, 30 and 100 μg min⁻¹ at 30 min intervals if BP remained ≥140/80 mmHg. If BP fell below 140/80 mmHg, the infusion rate was not increased and infusion continued at this rate for 1 h. If the mean arterial pressure (MAP) fell to <70 mmHg, infusion was stopped.

All women also received standard clinical treatment as governed by their attending clinical team. Medication taken concurrently with GSNO infusion is detailed in Table 1. During the GSNO infusion, antihypertensive medication was not taken, if orally administered, or was titrated to BP, if intravenously administered.

Urinary protein and creatinine
A urine sample was obtained from an indwelling urinary catheter at baseline, hourly during GSNO infusion and at 1 h postinfusion. Protein and creatinine concentrations were determined using the modified pyrogallol-red molybdate and modified Jaffe methods, respectively. Analyses were performed according to the manufacturer’s instructions using an automated Siemens Dimension RxL Max integrated clinical system (Siemens AG, Munich, Germany). Urinary protein-to-creatinine ratio (PCR) was calculated in grams per millimole.

Blood samples
Venous blood (20 ml) was drawn through an 18 gauge needle within 1 h prior to commencement of GSNO infusion. Immediately prior to the end of infusion, a further 20 ml of venous blood was obtained from the antecubital fossa contralateral to the infusion. Blood for platelet studies was collected in 3 ml tubes containing hirudin (final concentration 15 μg ml⁻¹; Verum Diagnostica GmbH, Munich, Germany) and blood for biomarker analysis was collected in 10 ml tubes containing EDTA (final concentration 1.8 mg ml⁻¹; Becton Dickson, Franklin Lakes, NJ, USA). Processing of samples began within 10 min of venepuncture.

Platelet activation by flow cytometry
One microlitre of fluorescein isothiocyanate-labelled anti-human P-selectin antibody (R&D Sytems, Minneapolis, MN, USA) was added to 44 μl of HEPES-buffered saline [0.14 mol l⁻¹ NaCl, 5 mmol l⁻¹ KCl, 1 mmol l⁻¹ MgSO₄ and 10 mmol l⁻¹ HEPES (sodium salt), pH 7.4]. Five microlitres of hirudinized whole blood was then added within 20 min, and samples were incubated at room temperature for 20 min. The reaction was stopped by 10-fold dilution in formal saline (0.2% formaldehyde in 0.9% NaCl). Negative controls for the P-selectin antibodies were set using an appropriate isotype control (mouse IgG1; R&D Systems). Samples were further diluted 10-fold in formal saline immediately prior to flow cytometry, which was carried out on an XL-MCL flow cytometer running EXPO 32 software, and data analysis was performed using CXP software (Beckman Coulter, High Wycombe, UK). Data were

Table 1
Baseline details for women undergoing GSNO infusion

<table>
<thead>
<tr>
<th>Infusion ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>Age (years)</td>
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<td>32</td>
<td>38</td>
<td>28</td>
<td>37</td>
<td>42</td>
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<tr>
<td>Parity</td>
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<td>0</td>
<td>29</td>
<td>34</td>
<td>26</td>
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<tr>
<td>BMI (kg m⁻²)</td>
<td>26</td>
<td>40</td>
<td>120/80</td>
<td>120/70</td>
<td>120/70</td>
<td>108/70</td>
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<tr>
<td>First trimester BP (mmHg)</td>
<td>100/64</td>
<td>125/70</td>
<td>100/60</td>
<td>150/106</td>
<td>154/90</td>
<td>162/113</td>
</tr>
<tr>
<td>Baseline BP prior to infusion (mmHg)</td>
<td>142/96</td>
<td>150/106</td>
<td>154/90</td>
<td>162/113</td>
<td>142/80</td>
<td>158/98</td>
</tr>
<tr>
<td>Medications concurrent with GSNO infusion</td>
<td>MgSO₄ iv</td>
<td>Labetalol po</td>
<td>Nifedipine LA po</td>
<td>MgSO₄ iv</td>
<td>Labetalol po</td>
<td>Nifedipine LA po</td>
</tr>
<tr>
<td>Proteinuria (g (24 h)⁻¹)</td>
<td>1.07</td>
<td>0.82</td>
<td>0.09</td>
<td>0.17</td>
<td>0.17</td>
<td>0.22</td>
</tr>
<tr>
<td>PCR prior to infusion</td>
<td>1.6</td>
<td>1.07</td>
<td>0.82</td>
<td>0.09</td>
<td>0.17</td>
<td>0.22</td>
</tr>
<tr>
<td>Proteinuria (g (24 h)⁻¹)</td>
<td>10.8</td>
<td>1.6</td>
<td>8.9</td>
<td>2.5</td>
<td>0.6</td>
<td>++</td>
</tr>
</tbody>
</table>

Abbreviations are as follows: BMI, body mass index; BP, blood pressure; GSNO, S-nitrosoglutathione; LA, long acting; iv, intravenous; PCR, protein-to-creatinine ratio; po, oral.
*Stopped during GSNO infusion. †Stopped prior to infusion.
recorded as the percentage of platelets expressing P-selectin (percentage positive).

**Plasma biomarkers**

Blood samples in EDTA were centrifuged at 1600g at 4°C for 20 min. Plasma was transferred into darkened Eppendorf tubes in 1.5 ml aliquots. Aliquots were snap-frozen in liquid nitrogen and stored at −80°C until required for analysis.

Assays for soluble fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) were performed using Meso Scale Discovery (MSD, Gaithersburg, MD, USA) electrochemiluminescence assays. Soluble endoglin (sEng) was measured by R&D Systems Quantikine ELISA kit. All assays were performed according to the manufacturer’s instructions. Quality control pools were run at the beginning and end of each batch. Samples were analysed in duplicate, and the mean concentration value was reported. Sample duplicates with coefficients of variation >10% were repeated.

**Statistical analysis**

Maternal and fetal cardiovascular and Doppler parameters were assessed using a Kruskal–Wallis test with post hoc analysis; pre- and post-GSNO infusion values of platelet P-selectin expression, urine PCR and soluble biomarkers were compared using a Wilcoxon signed-ranks test. A value of \( P < 0.05 \) was considered significant. Analysis was performed using MedCalc v12.3 (MedCalc Software, Mariakerke, Belgium).

### Results

Eighteen women were eligible, of whom 12 agreed to participate. Six women did not undergo infusion because immediate delivery was deemed necessary (three women), an exclusion criterion was met (one woman), intrauterine death occurred (one woman) or termination of pregnancy was performed (one woman). Six women with early-onset pre-eclampsia underwent GSNO infusion. One woman was infused on two occasions, 1 week apart, after agreement from the research ethics committee and data monitoring committee. In this case, data for the second infusion, whilst the woman was more severely pre-eclamptic, are included in the data analysis. Baseline demographic data are shown in Table 1.

Table 1 details concurrent medications. Three women were receiving intravenous MgSO\(_4\) (1 g h\(^{-1}\)) throughout the period of GSNO infusion; two were receiving intravenous labetalol infusion. However, in one case this was stopped 1 h prior to GSNO infusion; in the other case, the labetalol infusion was titrated and discontinued. Likewise, one woman was concurrently receiving intravenous hydralazine, which was titrated to her BP and as this fell during the GSNO infusion, the hydralazine was discontinued. Three women were receiving oral labetalol and four women oral nifedipine (in one case, concurrently).

Table 2 details the effect of GSNO infusion on cardiovascular and urinary parameters and biomarkers. Infusion of GSNO resulted in a reduction in AIx-75 from baseline.
The significant changes occurred at infusion rates of 30 and 100 μg min⁻¹ GSNO (mean reduction in AIx-75, −6 and −13%, respectively, P < 0.05; Figure 1).

Reductions in both diastolic (P = 0.017) and systolic central BPs (P = 0.008) were seen. The reduction in central BP was significant at 100 μg min⁻¹ GSNO (systolic BP, −26 mmHg, P < 0.05; and diastolic BP, −16 mmHg, P < 0.05). Likewise, MAP reduced with increasing GSNO infusion rate (P = 0.004); significantly so at 100 μg min⁻¹ GSNO (−19 mmHg, P < 0.05; Figure 2).

Peripheral systolic BP was unchanged (P = 0.20), but peripheral diastolic BP was reduced (P = 0.012). The reduction in peripheral diastolic BP at 100 μg min⁻¹ was significant from baseline (−15 mmHg, P < 0.05). No significant changes were found in central or peripheral pulse pressures (P = 0.18 and P = 0.34, respectively). Maternal heart rate remained constant (P = 0.55).

One woman declined aPWV measurement and another was too obese for the femoral cuff to fit. In the remaining four women, there was no significant change in aPWV (P = 0.21).

Platelet surface P-selectin expression was lower following GSNO infusion [mean (interquartile range) 6.3 (4.9–7.6) vs. 4.1 (3.1–5.7)% positive, Wilcoxon test P = 0.03; Figure 3]. Soluble endoglin showed a borderline significant reduction at maximal infusion rate [mean (interquartile range) 177 (144–295) vs. 158 (131–243) ng ml⁻¹, Wilcoxon test P = 0.06]. There were no differences in pre-/postinfusion VEGF, sFlt, PIGF or sFlt : VEGF or sFlt : PLGF ratios.

The range in PCR prior to infusion was large (0.08–0.88 g mmol⁻¹). The urinary PCR showed a borderline significant reduction at the conclusion of the GSNO infusion.
[mean (interquartile range) 0.37 (0.09–0.82) vs. 0.23 (0.07–0.49) g mmol⁻¹, Wilcoxon test, \( P = 0.06 \); Figure 4]. There was no relationship between the degree of reduction in urinary protein excretion and change in BP whilst the GSNO infusion was in progress (\( r = -0.55, P = 0.25 \)).

The fetal heart rate did not change during GSNO infusion (\( P = 0.90 \)), and there were no significant cardiotocograph changes. There were no changes in maternal uterine, fetal umbilical, middle cerebral or ductus venosus Doppler PI at any dose of GSNO.

### Neonatal and maternal outcomes

Whilst the primary purpose of the study was as a dose-ranging study for GSNO in early-onset pre-eclampsia, neonatal and maternal safety outcomes were collected as stipulated by the data monitoring committee. There was one neonatal death at 11 days of age due to extreme pre-maturity and growth restriction following delivery at 26 weeks of gestation of a neonate weighing 620 g (Table 3). Three women reported headache, which resolved with 1 g paracetamol administration. There were no maternal adverse outcomes.

### Discussion

We report a reduction in both arterial pulse wave reflection and platelet activation in women with early-onset pre-eclampsia receiving GSNO infusion. In addition to changes in Alx, we observed a borderline significant reduction in urinary protein excretion, expressed by PCR following GSNO infusion. Although a reduction in BP did occur throughout the infusion, this did not explain the effects of GSNO on protein excretion. It may that with six participants we are unable to demonstrate an association between BP reduction and a reduction in proteinuria. However, inhibition of NO synthesis is known to cause proteinuria, and dysfunction of NO pathways underlies the pathophysiology of pre-eclampsia. It is, therefore, plausible that replenishment of bioavailable NO by GSNO infusion reverses these processes and reduces proteinuria. This finding is entirely novel, and we are not aware that any agent has been shown to reduce PCR or protein excretion in pre-eclampsia; further study of this finding should be considered in future studies.

The Alx is generally considered a measure of pulse wave reflection, which is determined by small vessel tone and impedance, although alternative views are proposed and it may have its limitations, particularly in young healthy populations [16]. Small vessel tone is very sensitive to NO bioavailability and hence nitrates, which lower arterial impedance [17, 18]. Likewise, blocking of basal NO synthesis enhances impedance mismatch and, consequently, increases Alx [19]. The Alx is also a simple, reproducible, non-invasive technique that can be performed in a few minutes [14]. Gold-standard, non-invasive measures of endothelial function, such as flow-mediated dilatation, require both a longer time than Alx to perform and a high degree of participant co-operation by lying very still. This study was performed in a delivery unit with severely unwell patients around the time of preterm delivery of their babies, with multiple measurements required every 30 min; as such, a pragmatic approach was taken to endothelial function measurement and NO bioavailability, and we considered Alx to be a suitable surrogate.

Infusion of GSNO caused a significant reduction in central BP without changing peripheral BP. This is likely to be the result of the fall in wave reflection; the reflected wave contributes to central systolic pressure but rarely influences brachial pressure, because it arrives later in the cardiac cycle. The fall in central pressure may be beneficial, even in the absence of peripheral BP change, because it is

### Table 3

Clinical outcomes in women undergoing GSNO infusion

<table>
<thead>
<tr>
<th>Infusion ID</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
<tr>
<td>GSNO infusion to delivery (days)</td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gestation at delivery (weeks + days)</td>
<td>29(^{+2})</td>
<td>32(^{+5})</td>
<td>25(^{+5})</td>
<td>27(^{+3})</td>
<td>28(^{+6})</td>
<td>26(^{+2})</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>1060</td>
<td>1230</td>
<td>620</td>
<td>860</td>
<td>1009</td>
<td>860</td>
</tr>
<tr>
<td>Apgar score (at 1, 5 and 10 min)</td>
<td>68.9</td>
<td>88.9</td>
<td>78.9</td>
<td>35.7</td>
<td>26.8</td>
<td>66</td>
</tr>
<tr>
<td>Alive at discharge</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Figure 4**

Urine protein-to-creatinine ratio pre- and post-GSNO. Wilcoxon signed-rank test, \( P = 0.06 \). Graph shows pre- and post-GSNO values of each participant.

Gold-standard, non-invasive measures of endothelial function, such as flow-mediated dilatation, require both a longer time than Alx to perform and a high degree of participant co-operation by lying very still. This study was performed in a delivery unit with severely unwell patients around the time of preterm delivery of their babies, with multiple measurements required every 30 min; as such, a pragmatic approach was taken to endothelial function measurement and NO bioavailability, and we considered Alx to be a suitable surrogate.
central pressures to which vulnerable organs, such as the brain, cardiac left ventricle and kidneys, are exposed [20, 21].

In normotensive pregnancies, cardiac left ventricular mass increases. In pre-eclampsia, however, the increase in left ventricular mass is greater and the ejection fraction is reduced [22]. These changes are particularly notable in early-onset pre-eclampsia and may persist postpartum, after the pre-eclampsia has resolved [23]. Cardiovascular studies, such as CAFE [20] and REASON [21], have demonstrated that by reducing pulse wave reflection, central systolic BP is decreased to a greater extent than peripheral BP, and that this may reduce left ventricular mass. In particular, β-blockers seemed to be ineffective at reducing pulse wave reflection and left ventricular hypertrophy [21]. Further studies of prolonged infusion of GSNO are required to determine whether the effect of GSNO in reducing central BPs has any benefit on the cardiac changes seen in pre-eclampsia, or indeed other maternal outcomes.

In contrast to AIx, aPWV did not change during GSNO infusion. Aortic pulse wave velocity is a measure of aortic stiffness, which is largely governed by elastic elements within the wall rather than smooth muscle tone. In humans, nitro-vasodilators do not appear to alter aPWV independently of changes in MAP [24]. Moreover, in contrast to AIx, aPWV does not appear to be raised in women with pre-eclampsia, independently of BP [25]. This finding suggests that the effect of GSNO is preferentially an effect of reducing impedance in small to medium-sized arterial vessels and, hence, pulse wave reflection.

Platelet degranulation, as evidenced by surface CD63 [26] or P-selectin expression, is elevated in pre-eclampsia in comparison to normotensive pregnancies [1, 27]. We observed a significant reduction in platelet P-selectin expression following GSNO infusion, consistent with findings from a previous study [2, 5]. Separately, the use of GSNO in a case of postpartum Haemolysis, elevated liver enzymes and low platelet (HELLP) syndrome, a condition characterized by thrombocytopenia and microangiopathic haemolytic anaemia, resulted in a reduction in BP and increase in platelet count [3, 28]. The role of NO in determining the non-adhesive properties of the vascular endothelium [4, 29] is established. Release of NO from the endothelium can inhibit both platelet aggregation in response to platelet agonists and platelet adhesion to the extracellular matrix [30].

Activation of soluble guanylate cyclase is believed to be the primary route by which NO mediates its effects on platelet function [31]. However, there is recent evidence of a cGMP-independent effect of NO on platelet function [32, 33]. The mechanisms by which this occurs remain unclear, and effects via platelet-surface signalling [33], intracellular Ca2+ trafficking [32] and protein nitrosylation have been postulated [34]. S-Nitrosothiol (which include GSNO) in particular, appear also to act through cGMP-independent pathways [32, 33]. It has been suggested the GSNO has ‘platelet preferential’ actions [35]. This could, in part, be explained by GSNO being metabolized to NO more readily by platelets than in the vasculature [36], or by the cGMP-independent effects being more pronounced in platelets [33].

The finding that NO donors might have a therapeutic role in pre-eclampsia is not a new observation. Glycerol trinitrate is effective in controlling BP in severe pre-eclampsia [37] and reduces total peripheral vascular resistance in women with gestational hypertension when combined with oral hydration and antihypertensives [38]. Prolonged treatment of 12 pre-eclamptic women with the nitric oxide donor isosorbide mononitrate reduced BP and uteroplacental impedance [39], and similar effects were found with isosorbide dinitrate [40]. Previously, sodium nitroprusside has been widely used in pre-eclampsia, although any non-antihypertensive benefit that could be gained from its use as an NO donor is limited by it causing profound hypotension. In contrast, GSNO demonstrates potentially beneficial effects at doses that do not cause hypotension.

We report a reduction of borderline significance in levels of the vascular biomarker sEng, but no change in sFlt-1, VEGF or PlGF levels. This effect on sEng during GSNO infusion is consistent with in vitro studies showing that glycerol trinitrate can reduce the hypoxia-mediated release of sEng from placental explants [41].

Several considerations are pertinent to interpretation of these data. Three women were receiving MgSO4, which has been shown to reduce AIx at higher doses than we were administering [42]. However, MgSO4 infusion was already established prior to commencement of GSNO, and GSNO caused a reduction in AIx from this baseline. Given the consistent dose-dependent responses to GSNO seen in all women and rapid return to baseline values after cessation of the infusions, including those in women with ongoing MgSO4, a genuine effect on AIx can be attributed to GSNO.

As mentioned previously, for pragmatic reasons, in this study we did not measure endothelial function directly. The transfer function for AIx has not been validated in pregnancy; however, given the invasive techniques required, validation is unlikely to be performed. Likewise, given the constraints of a study involving severely ill women requiring intensive treatment and monitoring prior to preterm delivery, detailed cardiovascular testing was not performed. This should be considered in future studies when more prolonged infusion at the dose increments is being performed.

The study is a small, preliminary uncontrolled study, which offered recruitment only to those women with early-onset pre-eclampsia, and at least one-half of these women required imminent delivery before it was possible to commence the infusion. S-Nitrosoglutathione is a temperature- and light-sensitive substance that, in this context, requires intravenous infusion. As such, there
are practical considerations that would surround its therapeutic use. However, if future clinical trials demonstrated benefit to either mother or fetus, then prolonged infusions would be feasible under intensive monitoring. Recently, orally administered GSNO reductase inhibitors have been developed and used in animal models [43, 44]. This could provide an interesting alternative means to increase the GSNO levels in women with pre-eclampsia and circumvent some of the difficulties in storage and administration of GSNO.

Whilst GSNO has previously been shown to reduce BP in 10 women with severe pre-eclampsia, its effects on pulse wave reflection, central BP, proteinuria and soluble factors have not previously been investigated. S-Nitrosogluthathione caused a dose-dependent reduction in arterial pulse wave reflection at doses lower than those that had an effect on BP. The nonhaemodynamic effects of reducing platelet activation and improvement in both proteinuria and sEng occur at GSNO doses that do not significantly affect BP. The reduction in proteinuria would be of clinical importance and, to our knowledge, has not been documented with any agent used in the treatment of pre-eclampsia.

As no therapy exists that targets the disease process itself, the possible beneficial renal and platelet effects observed suggest that the action of GSNO is not simply vascular, but that it may be targeting the underlying pathophysiology of the clinical manifestations. This therapeutic potential warrants early-phase clinical studies powered for prolongation of gestation, maternal morbidity and neonatal morbidity and mortality.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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