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Preeclampsia

L-(+)-Ergothioneine Significantly Improves the Clinical Characteristics of Preeclampsia in the Reduced Uterine Perfusion Pressure Rat Model

Rachel D. Williamson, Fergus P. McCarthy, Samprikta Manna, Emer Groarke, Douglas B. Kell, Louise C. Kenny, Cathal M. McCarthy

Abstract—Preeclampsia is a multifactorial hypertensive disorder of pregnancy founded on abnormal placentation, and the resultant placental ischemic microenvironment is thought to play a crucial role in its pathophysiology. Placental ischemia because of fluctuations in the delivery of oxygen results in oxidative stress, and recent evidence suggests that mitochondrial dysfunction may be a prime mediator. However, large clinical trials of therapeutic antioxidants such as vitamins C and E for the treatment of preeclampsia have been disappointing. L-(+)-ergothioneine (ERG)—an unusual amino acid betaine derived from histidine—has important cytoprotective and antioxidant properties under conditions of high oxidative stress. In this study, we investigated the potential therapeutic effects of administration of ERG in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia. ERG (25 mg/kg per day) was administered to rats on gestational day 11. On gestational day 14, RUPP surgery was performed, and on gestational day 19, blood pressure (mean arterial pressure) and fetal growth were measured. Production of mitochondria-specific H_2O_2 was analyzed in vivo in kidney samples. ERG ameliorated the hypertension (129±3 versus 115±4 mm Hg; P=0.01; n=8) and significantly increased pup weight in RUPP rats. ERG also significantly decreased circulating levels of antiangiogenic sFlt-1 (soluble fms-like tyrosine kinase-1) in RUPP rats (1367±245 pg/mL; P=0.04). Mitochondria-specific H_2O_2 (0.022±0.003 versus 0.029±0.001; MitoP/B ratio, n=3; P=0.05) was also significantly decreased in kidney tissue in RUPP rats treated with ERG. These data support the potential use of ERG for the treatment of preeclampsia. (*Hypertension*. 2020;75:561-568. DOI: 10.1161/HYPERTENSIONAHA.119.13929.) • Online Data Supplement

Key Words: dietary supplements ■ humans ■ oxygen ■ preeclampsia ■ vitamins

Preeclampsia is a multisystemic disorder of pregnancy characterized by high blood pressure at or after 20 weeks of gestation accompanied by and/or proteinuria, acute kidney injury, liver dysfunction, or fetal growth restriction.[1] It affects >8 million pregnancies worldwide annually and is the leading cause of maternal death.[2] Despite extensive research, the exact pathophysiological mechanisms underlying this syndrome remain poorly elucidated. Nonetheless, defective placentation is strongly considered to be a critical event in the pathology of the disorder.[2] Failure to remodel spiral arteries results in high-pressure blood flow–mediated placental damage and intermittent fluctuations in oxygen delivery, which exposes the placenta to oxidative stress.[3] The resultant placental ischemic microenvironment is inherently linked to increased production and secretion of deleterious soluble mediators that provoke extensive maternal inflammation and endothelial dysfunction.[4] Several reports have observed higher levels of markers of oxidative stress (including F_2-isoprostanes, nitrotyrosine,

and 4-hydroxynonenal staining) in placental tissue from preeclamptic pregnancies compared with those from uncomplicated pregnancies.[5–8]

While there are a number of different cellular sources of reactive oxygen species (ROS), mitochondria are the major cellular producers.[9] Furthermore, in terms of preeclampsia, there is growing evidence incriminating mitochondrial dysfunction in its underlying pathophysiology.[10] Initial studies showed increased mitochondrial lipid peroxidation and enhanced susceptibility to oxidative damage in placental tissue of pregnancies complicated by preeclampsia.[11] More recently, work has confirmed this association with strong evidence of perturbation of mitochondrial function in the metabolite profile of plasma samples taken at 15 weeks of gestation from patients who subsequently developed preeclampsia.[12]

While there is significant evidence for the pathogenic role of oxidative stress in the development of preeclampsia, clinical trials of antioxidant interventions were disappointing and

not clinically effective in treating the disorder. One plausible explanation is that these antioxidants missed the intracellular location of ROS production, namely the mitochondria; hence they have failed to alleviate the pathological oxidative damage. Another is that molecules such as L-ascorbate can actually be pro-oxidant in the presence of free iron.[9] L-ergothioneine (ERG) is an unusual thiohistidine betaine amino acid and is a naturally occurring antioxidant discovered over a century ago in the rye ergot.[13–16] The predominant role of ERG, via a variety of mechanisms, is to serve as an antioxidant and cellular protectant against various kinds of ROS.[17] Additionally, there has been some circumstantial evidence that ERG could target mitochondria[18] and hence could dampen exaggerated mitochondria-specific ROS (mROS) in response to oxidative stress.

Therefore, we aimed to investigate the role of ERG as a potential therapeutic target for preeclampsia using the reduced uterine perfusion pressure (RUPP) model in pregnant rats. The placental ischemic RUPP model has numerous features of preeclampsia that are clinically evident in women and has been used as a preclinical model for the investigation of novel therapeutic targets for the treatment of preeclampsia.[19,20] Moreover, we additionally wanted to examine whether ERG ameliorated the clinical characteristics of preeclampsia, in part, via regulation of mROS production. To this end, we used a novel ratiometric mass spectrometry probe MitoB that specifically accumulates in mitochondria and generates a MitoP phenol product on reaction with H_2O_2, which can subsequently be analyzed ex vivo by mass spectrometry.

Materials and Methods

The techniques and data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Sprague Dawley-timed pregnant rats were supplied and maintained by the University College Cork Biological Services Unit. Animals were maintained at a temperature of 21°C, with a 12-hour light/dark cycle and free access to food and tap water. All the procedures were performed in accordance with the National Guidelines and the European Directive 2010/63/EU, under an authorization issued by the Health Products Regulatory Authority Ireland and approved by the Animal Ethics Committee of University College Cork (AE19130/P037).

RUPP Procedure

The RUPP procedure is a well-established surgical model for studying the link between placental ischemia and hypertension in the pregnant rat and has been previously described in detail.[19,21] In brief, on gestational day (GD) 14, under isoflurane anesthesia, RUPP reduction in blood flow to the uteroplacental unit was achieved by placing a silver clip (0.2 mm ID) on the abdominal aorta (1 clip) above the iliac bifurcation. Two further clips (0.1 mm ID) were carefully placed around the left and right ovarian arteries. Sham surgery was performed as controls, which involved abdominal incision but did not involve insertion of any clip on either abdominal aorta or ovarian arteries. On GD18, a chronic indwelling catheter was inserted into the carotid artery, and on GD19, mean arterial blood pressure was recorded in conscious animals.

ERG In Vivo Experimental Protocol

Four experimental groups were used to investigate the effect of administration of ERG in the RUPP rat model of preeclampsia. Pregnant rats were divided into Sham (n=8), Sham+ERG (n=8) or RUPP (n=8), and RUPP+ERG (n=8). ERG was administered at 25 mg/kg per day in their drinking water on GD11 until the end of the experiment on GD19. The dose for ERG (25 mg/kg per day) was selected based on previously published rodent studies using this antioxidant.[22,23] ERG was provided by Tetrahedron (Paris, France; www.tetrahedron.fr).

Plasma Collection

Blood collected from EDTA vacutainers was centrifuged at 2000*g* and 2400*g* for 10 minutes at 4°C; plasma was removed and stored at −80°C for further analysis.

Urine Analysis

On GD18, each rat was singularly housed in a metabolic cage and urine collected overnight. All samples were stored immediately following collection at −80°C. Albumin:creatinine ratios were calculated after measurement of albumin using an immunoturbidimetric test for the quantitative determination of albumin in an OLYMPUS AU5832 analyzer and urine creatinine using a kinetic colorimetric test (Jaffé method).[24] Similarly, protein:creatinine ratios were calculated after measurement of protein by adding benzethonium chloride, which resulted in the formation of a fine suspension, which was then quantified turbidimetrically at 525 nm using an OLYMPUS analyzer.

Measurement of Antiangiogenic Protein sFlt-1 by ELISA

Circulating sFlt-1 concentration in plasma samples from all experimental groups was quantified by ELISA using a Quantikine sFlt-1 immunoassay (R&D Systems) as per manufacturers' instructions.

Isolation of RNA and Real-Time Polymerase Chain Reaction Analysis

RNA was extracted from placental tissue using the Trizol method. SLC22A4 (ERG transporter) should be replaced with OCTN1 (organic cation transporter, novel, type 1), SOD1 (superoxide dismutase 1), SOD2 (superoxide dismutase 2), and UCP-1 (uncoupling protein-1), PCG-1α (peroxisome proliferator activated receptor-γ coactivator 1α) and Nrf2 (nuclear factor [erythroid-derived 2]–like 2) gene expression was quantified by real-time polymerase chain reaction using StepONE Plus Detection system. Taqman assays (Applied Biosciences) and Sybr Green primers were used for quantification. The amounts of the target genes were normalized to the geometric mean of internal control gene 18S and were determined using the comparative $2^{-\Delta\Delta CT}$ method.

Isometric Myography

In all groups, third-order mesenteric arteries were dissected and mounted on a 4-channel wire myograph (Model 610 mol/L Danish Myo Technology) containing oxygenated (95% O_2 and 5% CO_2) physiological salt solution at 37°C. Vessels were normalized to achieve a transmural pressure of 100 mmHg using the DMT Normalization software. Isometric tension was recorded and displayed using Powerlab Chart Software (AD Instruments). The viability of the smooth muscle was examined by the addition of a 123-mmol/L KCl solution. After physiological salt solution washes, concentration responses were performed with thromboxane mimetic U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$; 10^{-9} to 10^{-5} M) and either BK (bradykinin; 10^{-8} to 10^{-5} M), acetylcholine (10^{-8} to 10^{-5} M), or sodium nitroprusside (10^{-8} to 10^{-5} M), respectively.[25,26]

Estimation of Mitochondrial H_2O_2 in the RUPP Model In Vivo

Mitochondrial hydrogen peroxide was measured in vivo using the Cayman Chemical Hydrogen Peroxide Ratiometric MaxSpec kit based on the MitoB mass spectrometric probe method described previously.[27] Briefly, 75 nmol MitoB in 50 μL saline was administered by tail-vein injection to rats in the 4 experimental groups on GD19, 4 hours before the end of the experiment. At the end of the procedure, kidney tissues were dissected out, snap-frozen, and stored at −80°C. For mitochondrial H_2O_2 analysis, kidney tissues were homogenized,

spiked with deuterated internal standards, and MitoB, and its product MitoP was extracted using acetonitrile/formic acid. MitoB and MitoP present in kidney tissue were measured using UPLC Xevo TQD mass spectrometer (Waters), and the amounts of MitoP and MitoB in each sample were determined relative to a standard curve. The MitoP/B ratios for each sample were then calculated.

Statistical Analysis

All data are expressed as mean±SEM or fold change relative to control. Analysis was performed using GraphPad Prism, and Student t test was applied when comparisons were made between 2 groups. For analyzing the differences in real-time polymerase chain reaction and MitoB/P ratio, a 2-way ANOVA was run followed by Bonferroni post hoc test.

Results

ERG Ameliorated RUPP-Induced Hypertension

Mean arterial blood pressure was significantly increased in the RUPP group compared with sham group (129±3 versus 117±7 mmHg; P=0.05; n=8; Figure 1A). Furthermore, administration of ERG significantly reduced mean arterial blood pressure in the RUPP rats (129±3 versus 115±4 mmHg; P=0.01; n=8; Figure 1A). Mean arterial blood pressure in sham rats treated with ERG was not significantly different from RUPP rats treated with ERG, indicating the beneficial effect of ERG antioxidant in reducing blood pressure occurs in response to placental ischemia. There was no significant difference in either the microalbumin:creatinine ratio (Figure 1B) or the protein:creatinine ratio (Figure 1C) in the RUPP group compared with Sham group, nor was there a significant difference in the microalbumin:creatinine ratio in the RUPP rats treated with ERG.

ERG Improves Fetal Weight

Fetal birth weight was significantly decreased in the RUPP group compared with the Sham group (1.8±0.04 versus 2±0.03 g; P=0.0004; Figure 2A). Administration of ERG significantly rescued fetal growth restriction in the RUPP rats (2±0.03 versus 1.8±0.04 g; P=0.0006; Figure 2A). Pup weight was not significantly different between Sham or RUPP rats treated with ERG (2±0.03 versus 2±0.03 g; P=0.78; Figure 2A), indicating the beneficial effect of ERG in rescuing fetal weight during placental ischemia. Placental weights were significantly reduced in RUPP group compared with Sham group (0.4±0.01 versus 0.5±0.01 g; P=0.002; Figure 2B).

The administration of ERG had no effect on placental weight in the RUPP rats (0.4±0.01 versus 0.4±0.01 g; Figure 2B). A significant decrease in pup number was observed in the RUPP group compared with Sham group (11±1 versus 14±1; P=0.02; Figure 3C). There was no significant difference in pup number in RUPP rats treated with ERG relative to RUPP rats (13±2 versus 11±1). There was no significant difference in the crown-to-rump length of pups (Figure S1 in the online-only Data Supplement) or abdominal circumference (Figure S2) in any of the studied groups. Finally, maternal weight did not differ between any of the studied groups (Figure S3).

ERG Reduces Circulating sFlt-1 Levels

There was a significant increase in the circulating soluble antiangiogenic mediator sFlt-1 in the RUPP group compared with the sham group (1995±97 versus 1185±349 pg/mL; P=0.04; Figure 3). Administration of ERG significantly decreased circulating sFlt-1 levels in RUPP rats (1367±245 pg/mL; P=0.04; Figure 3).

Effect of ERG on Vasorelaxation in the RUPP Model

Mesenteric arteries from the RUPP group displayed impaired vasorelaxation in response to BK when compared with the sham group (R_{max}: 28±7% versus 52±9%, P=0.01; log Half maximal effective concentration EC50: −6.6±0.2 versus −6.8±0.3 mol/L, P=0.7; Figure 4A). However, no significant differences were seen in the vascular response to the endothelial independent vasodilator sodium nitroprusside (R_{max}: 78±5% versus 80±5%, P=0.36; log EC50: −7.1±0.2 versus −7.2±0.4 mol/L, P=0.9; Figure S4) or acetylcholine (R_{max}: 83±8% versus 81±6%, P=0.1; log EC50: −7.1±0.2 versus −7±0.1 mol/L, P=0.6; Figure S5). Treatment with ERG had no significant effect on the vasorelaxant responses of mesenteric vessels in response to BK (R_{max}: 23±5% versus 28±7%, P=0.9; log EC50: −6.7±0.2 versus −6.8±0.2 mol/L, P=0.7; Figure 4B) or sodium nitroprusside (R_{max}: 78±6% versus 74±4%, P=0.8; log EC50: −7.1±0.2 versus −7.2±0.2, P=0.9; Figures S6 and S7) in RUPP rats.

ERG Alters Placental Expression of Markers of Mitochondrial ROS-Detoxifying Enzymes

Placental expression of mitochondrial orchestrator PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α;
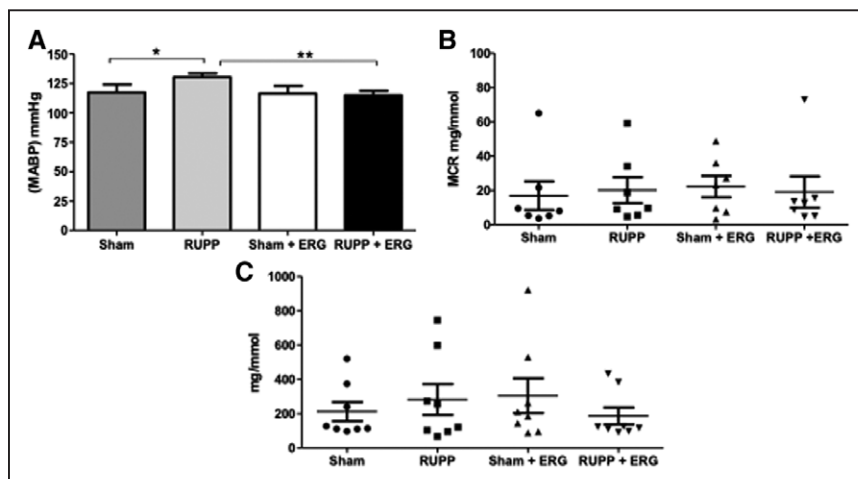
Figure 1. L-ergothioneine (ERG) attenuates blood pressure in reduced uterine perfusion pressure (RUPP) rats. RUPP rats (n=8) showed (A) significantly elevated mean arterial blood pressure (MABP) compared with sham group (n=8). ERG (25 mg/kg per d) reduced MABP in RUPP rats (n=8). Data are presented as mean±SEM. *P<0.05, **P<0.01. **B**, ERG did not alter microalbumin to creatinine ratio (MCR) in RUPP rats: administration of ERG (25 mg/kg per d) did not significantly affect MCR in RUPP rats. **C**, ERG did not alter protein:creatinine ratio in RUPP rats: administration of ERG (25 mg/kg per d) did not significantly affect MCR in RUPP rats.
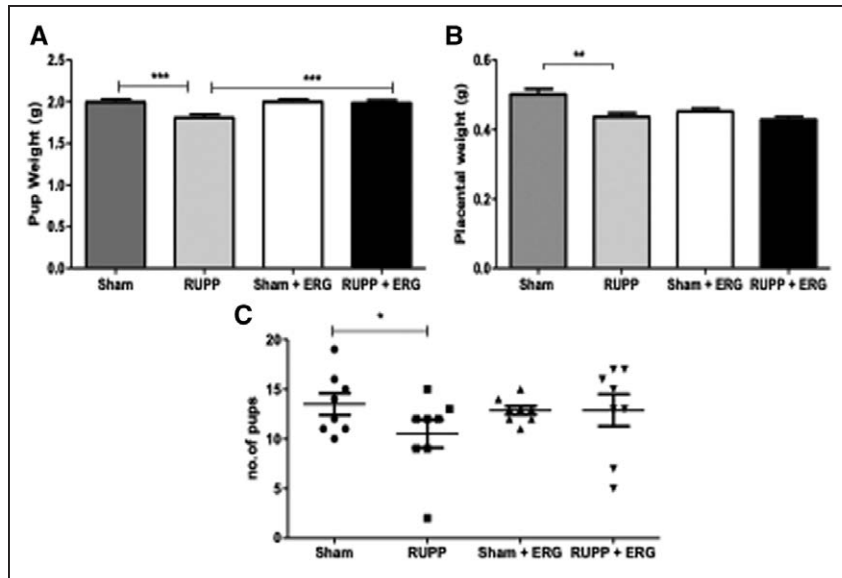
Figure 2. L-ergothioneine (ERG) improves fetal outcomes in reduced uterine perfusion pressure (RUPP) rats. RUPP rats (n=8) show reduced (**A**) pup weight and (**B**) placental weight and (**C**) litter size compared with sham group (n=8). Administration of ERG (25 mg/kg per d) improved fetal outcome in RUPP rats. Data are presented as mean±SEM. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

1±0.4-fold versus 0.4±0.5-fold; n=8; $P=0.01$; Figure 5A) and mitochondrial ROS-detoxifying enzymes including UCP-1 (1±0.5-fold versus 0.2±0.5-fold; $P=0.04$) were significantly decreased in the RUPP rats. Nrf2—a transcriptional regulator of the mitochondrial antioxidant defence system and coactivated by PGC-1α—was also significantly reduced in the RUPP rats (1±0.2-fold versus 0.4±0.4-fold; n=8; $P=0.01$; Figure 5A). Placental expression of SOD1 antioxidant was significantly increased (1±0.3-fold versus 1.4±0.3-fold; n=8; $P=0.02$; Figure 5B), whereas mitochondrial SOD2 antioxidant was significantly reduced (1±0.3-fold versus 0.6±0.3-fold; n=8; $P=0.01$; Figure 5B) in RUPP rats compared with sham group.

ERG treatment significantly increased placental expression of PGC-1α (1.7±0.3-fold versus 1±0.5-fold; n=8; $P=0.02$), UCP-1 (4.9±0.6-fold versus 1±0.5-fold; n=8; $P=0.001$), and Nrf2 (1.6±0.3-fold versus 1±0.4-fold; n=8; $P=0.02$; Figure 5A) in RUPP rats. ERG treatment significantly increased placental expression of SOD2 in RUPP rats (1.5±0.3-fold versus 1±0.3-fold; n=8; $P=0.02$; Figure 5B). ERG treatment significantly increased both SOD1 (3.4±0.4-fold versus 1±0.8-fold) and SOD2 (1.4±0.3-fold versus 1±0.8-fold) expression in RUPP rats compared with sham rats. ERG treatment also significantly increased placental expression

of the ERG transporter OCTN1 (organic cation transporter, novel, type 1) (1.9±0.3-fold versus 1±0.5-fold) in RUPP rats compared with sham rats (Figure 5B).

ERG Reduces Mitochondria-Specific H_2O_2 Production in the Kidney

MitoP/B ratio was increased in kidney tissue of RUPP rats compared with the sham group (0.029±0.001 versus 0.024±0.006; n=3; $P=0.3$; Figure 6), indicating for the first time that mitochondria-specific H_2O_2 is increased in vivo as a result of placental ischemia. MitoP/B ratio was significantly increased in kidney tissue between RUPP and Sham rats treated with ERG (0.022±0.003 versus 0.017±0.003; n=3; $P=0.05$; Figure 6). Furthermore, pretreatment with ERG significantly reduced the MitoP/B ratio in kidney tissue in RUPP rats (0.022±0.003 versus 0.029±0.001; n=3; $P=0.05$; Figure 6), establishing that ERG reduces mitochondria-specific H_2O_2 production in vivo.

Discussion

Despite a significant amount of evidence for the pathological role of oxidative stress in the development of preeclampsia, clinical data from 2 major antioxidant vitamin trials have been negative.[28,29] One possible reason for these negative findings may be

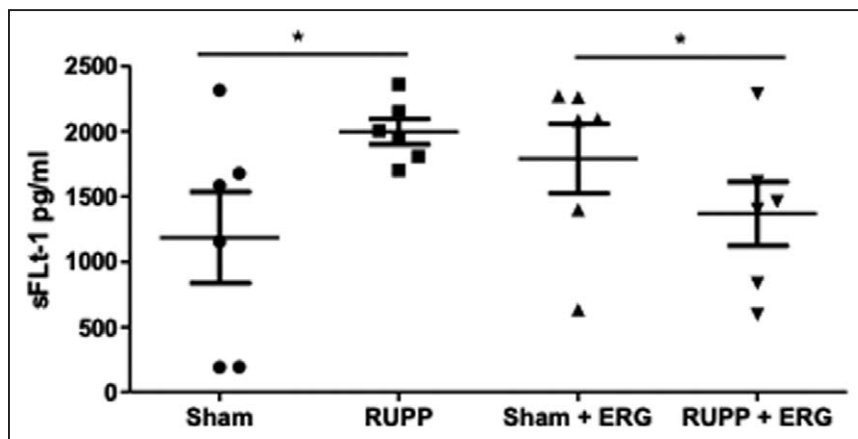**Figure 3.** L-ergothioneine (ERG) reduces circulating antiangiogenic factor sFlt-1 (soluble fms-like tyrosine kinase-1) in reduced uterine perfusion pressure (RUPP) rats: circulating levels of sFlt-1 were significantly increased in RUPP rats (n=8) compared with sham controls (n=8). Administration of ERG significantly reduced circulating sFlt-1 levels in RUPP rats. Data are presented as mean±SEM. *$P<0.05$.
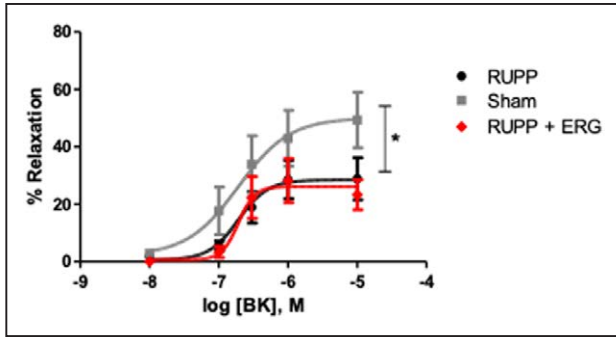
Figure 4. Impaired vasorelaxation in mesenteric arteries in reduced uterine perfusion pressure (RUPP) rats: RUPP rats (n=8) showed significantly impaired vasorelaxation in response to BK (bradykinin) compared with the sham group (n=8). Administration of L-ergothioneine (ERG) did not attenuate the impaired vasorelaxation to BK. Relaxation is calculated as a percentage of the maximum contraction and expressed as mean±SEM. *P<0.05.

due to the fact these interventions missed the primary intracellular producer of ROS, namely the mitochondria, or because in the presence of free or poorly liganded iron, substances such as ascorbate are actually pro-oxidant.[9] In the present study, ERG—an amino acid with potent antioxidant properties—attenuated hypertension and rescued fetal growth restriction in the preclinical RUPP rat model of preeclampsia, which closely mimics many aspects of preeclampsia during human pregnancy. Additionally, this study also reported the novel finding of reduced mitochondrial H_2O_2 levels in vivo following ERG administration. This work has highlighted that ERG acts as a potent antioxidant that ameliorates a number of phenotypic features of preeclampsia in a preclinical model of disease and mediated, in part, by the reduction of mROS. This work consequently proposes the potential of ERG as a viable therapeutic for the prevention of preeclampsia.

ERG is a water-soluble amino acid that is derived entirely from dietary sources. It has garnered much attention

recently as a potential therapeutic intervention,[13,30–33] partly because of its preferential accumulation within tissues undergoing significant oxidative stress. A recent study investigating the reproductive safety profile of ERG in pregnant Sprague Dawley rats established that ERG was well tolerated and with no adverse effects on a number of parameters (number of mating days, gestation length, pup viability index, or litter parameters).[34] Furthermore, ERG treatment of diabetic rats significantly improved embryo formation and quality.[31] Collectively, these data suggest ERG treatment may be safe to use in pregnancy and may prevent embryo malformations mediated by oxidative stress early in pregnancy. Consequently, we decided to administer ERG at an early point in pregnancy (GD11) in the preclinical RUPP model of preeclampsia.

The therapeutic effects of ERG in response to induced tissue damage in models of ischemia-reperfusion injury in the liver[35] and intestine[22] have previously been reported by the dampening of markers of oxidative stress and inflammation. Our novel data using ERG reported a reduction in hypertension and an improvement in fetal weight in response to placental ischemia in the preclinical RUPP model of preeclampsia, in part, due to regulation of mROS. Recent evidence identified a prominent role for mROS in modulating hypertension.[36] Using 2 in vivo murine models of hypertension (Ang II [angiotensin II] induced and deoxycorticosterone acetate salt), this group established that using a mitochondria-targeted antioxidant (Mito-Tempo) alleviated endothelial dysfunction, reduced vascular mitochondrial superoxide and subsequent hypertension.[36] Furthermore, similar to our work, Vaka et al[37] showed in the preclinical RUPP model that placental ischemia dysregulated mitochondrial function with elevated mROS and identified that treatment with mitochondria-targeted antioxidants attenuated hypertension with improvement in fetal outcomes in treated RUPP rats.
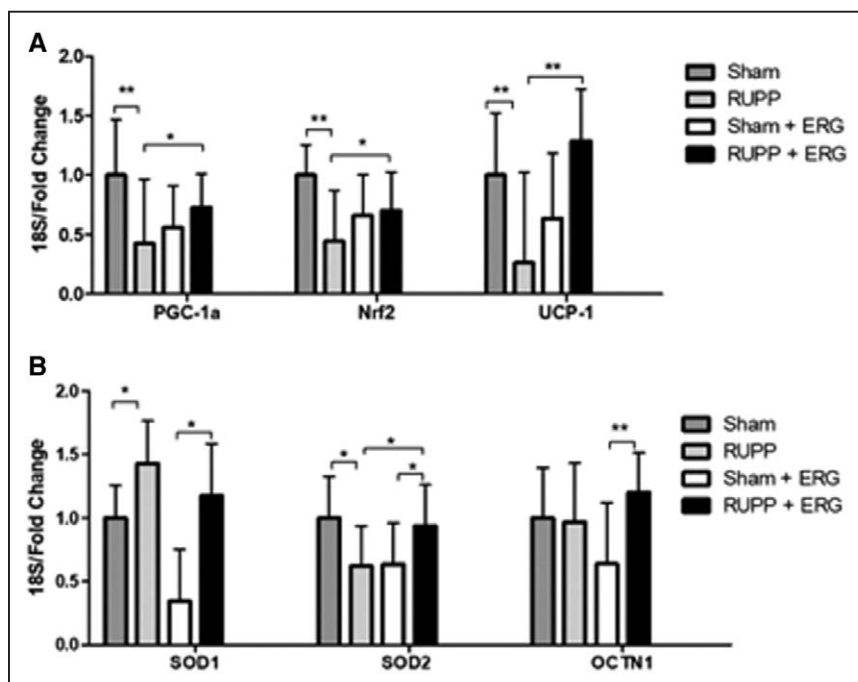

Figure 5. L-ergothioneine (ERG) regulates placental expression of mitochondrial ROS-detoxifying enzymes in reduced uterine perfusion pressure (RUPP) rats. (**A**) Placental expression of PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α), UCP-1 (uncoupling protein-1), and Nrf2 (nuclear factor [erythroid-derived 2]–like 2) expression were significantly reduced in RUPP rats (n=8) compared with sham group (n=8). Administration of ERG significantly increased placental expression of all these mitochondrial markers in RUPP rats. **B**, Placental expression of antioxidant SOD1 (superoxide dismutase 1) was significantly increased, whereas mitochondrial SOD2 (superoxide dismutase 2) expression was significantly decreased in the RUPP group compared with sham group. Administration of ERG significantly increased mitochondrial antioxidant SOD1 expression in RUPP rats compared with sham, while also significantly increased SOD2 expression in RUPP rats. Administration of ERG significantly increased OCTN1 (organic cation transporter, novel, type 1) expression in RUPP rats compared with sham. Data are presented as mean±SEM. *P<0.05, **P<0.01.
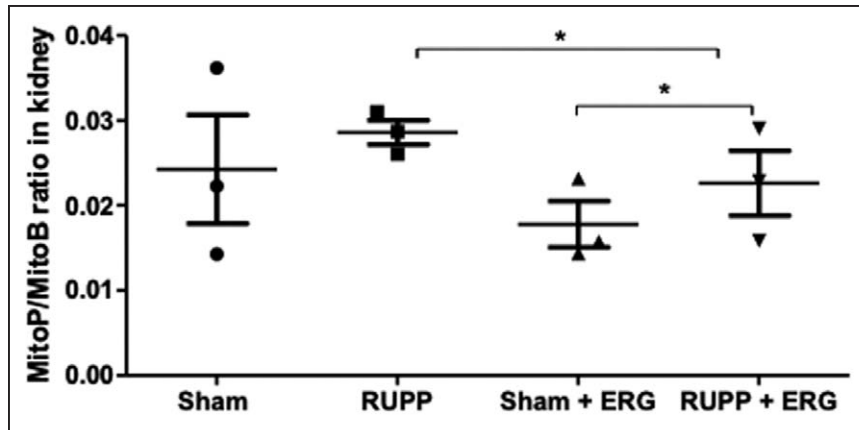
Figure 6. L-ergothioneine (ERG) reduces mitochondria-specific H_2O_2 levels. Rats were injected with MitoB for 4 h, and the ratios of MitoP/B were determined by Liquid Chromatography with tandem Mass Spectrometry. Data are represented as mean±SEM of 3 samples per group and corrected for ratio. Statistical analysis determined by a 2-way ANOVA followed by Bonferroni post hoc test was applied.*$P<0.05$. RUPP indicates reduced uterine perfusion pressure.

In this study, we found there was no significant increase in urinary protein in the RUPP rats compared with sham rats, which has also been reported in a recent RUPP study.[38] It could be proposed that the short duration of gestation in the rat decreases the prospect of inflicting severe renal damage to routinely detect proteinuria in these rats. Deleterious circulating mediators including sFlt-1 are secreted in response to placental ischemia and have devastating consequences on the maternal vasculature. Furthermore, we previously detected increased levels of mitochondria-specific superoxide production in human umbilical vein endothelial cells incubated with plasma from women with preeclampsia compared with matched controls and nonpregnant controls.[39] Additionally, Zsengeller et al[40] established an inverse correlation between placental sFlt-1 and mitochondrial complex IV, suggesting that sFlt-1 may be harmful to mitochondria in preeclampsia. In our study, ERG treatment reduced the circulating levels of sFlt-1 in RUPP rats, indicating that ERG may preserve mitochondrial function, in part, by reducing the availability of sFlt-1 to induce mitochondrial damage. The reduction in sFlt-1 as a result of ERG treatment did not result in a subsequent reduction in maternal vascular dysfunction in mesenteric arteries of the RUPP rats; however, future work could investigate maternal vascular dysfunction in a more physiologically relevant vasculature such as the uterine arteries.

The capacity to accurately measure the concentration of ROS, in particular mROS in vivo, has proved to be extremely challenging, yet is essential in understanding their physiological roles in certain diseases. The detection of mROS such as superoxide using fluorescent probes can be inferred, with certain caveats, by determining the changes in fluorescence or ex vivo tissue measurements of electron transport chain complexes, but these methods can be less selective and sensitive. One potential alternative is to use exogenous ratiometric probes (MitoB) injected into the animal models, which readily accumulate in mitochondria of tissues because of its possession of a triphenylphosphonium cation and which reacts with H_2O_2 in vivo to produce a diagnostic exomarker (MitoP) that can be accurately quantified by mass spectrometry.[41]

In this study, we have shown for the first time that this method of measuring mitochondrial H_2O_2 production in vivo is applicable in a rat model, without any adverse effects on reproductive safety. Furthermore, we reported that mitochondrial H_2O_2 was increased in kidney tissue in the RUPP group compared with the sham group. This is in agreement with recent work by Vaka et al,[37] who showed an increase in mROS in kidney tissue in the RUPP group by examining electron transport chain activity and cellular respiration ex vivo using isolated mitochondria from kidney tissues.[37] More importantly, treatment with ERG resulted in a significant reduction in mitochondria-specific H_2O_2 production in kidney tissue in RUPP rats, indicating that ERG may be mediating its therapeutic effects, in part, by directly reducing mROS production in the preclinical model of preeclampsia.

PGC-1α is a transcriptional coactivator that orchestrates a number of mitochondrial functions including antioxidant defense system and oxidative metabolism.[42,43] Consequently, PGC-1α directs the expression of mitochondrial ROS-detoxifying enzymes including superoxide dismutases (SOD1 and SOD2) and UCP-1, which are directly induced by this transcriptional orchestrator and Nrf2 nuclear receptor.[44] UCP-1 is a mitochondrial transporter with a well-defined role in regulating adaptive thermogenesis[45]; more recently, it has been shown to mediate inflated mROS production, in part, via mild uncoupling.[46] Further evidence to support our theory is provided by the fact that placental expression of markers of mitochondrial ROS-detoxifying enzymes and their transcriptional regulators were significantly improved in ERG-treated RUPP rats, in contrast to their reduced expression in RUPP rats.

At present, only one study has investigated the pharmacokinetics of ERG administration in human subjects.[30] ERG was rapidly absorbed and retained within the tissue/plasma with relatively low urinary excretion (<4% of administered dose). This work was further extended by Tang et al, who established that ERG and its metabolites are widely distributed in various tissues in male mice administered ERG over a range of days. This group also established that ERG was highly retained in the body and suggested this could be as a result of possible reabsorption by the kidneys.[47]

This study provides evidence of the therapeutic potential of ERG in a preclinical model of preeclampsia. ERG improved both hypertension and fetal weight in the RUPP rat model of preeclampsia. Furthermore, ERG treatment significantly altered mitochondrial function in both the kidney and placental tissue, which may, in part, be responsible for the beneficial effects on phenotypic features of preeclampsia in the RUPP model. Given its favorable safety profile, its long half-life and resistance to auto-oxidation and multiple mechanisms of action, not least its ability to regulate mitochondrial function, further studies are needed to explicitly define the protective mechanisms of ERG in treating preeclampsia in humans.

Perspectives

The causes of preeclampsia have remained an enigma despite intense research efforts in recent decades. Consequently, effective interventions and treatments remain elusive, and currently, there is no effective treatment for preeclampsia. Overall, this study provides evidence that exaggerated mROS can play a pathological role in the development of hypertension in response to placental ischemia in a preclinical rat model of preeclampsia. Additionally, this study demonstrates that ERG—a nutraceutical antioxidant—ameliorates some of the phenotypic features of preeclampsia in a preclinical model of disease, in part, by the reduction of mROS. This work opens a new avenue of investigations for new therapeutic options for preeclampsia.

Disclosures

D.B. Kell is a named inventor on a patent application involving the biotechnological production of L-(+)-ergothioneine in yeast. The other authors report no conflicts.

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Novelty and Significance

What Is New?

- There is growing evidence implicating mitochondrial dysfunction in the pathophysiology of preeclampsia.
- The nutraceutical antioxidant L-ergothioneine reduces blood pressure and rescues fetal growth restriction in the reduced uterine perfusion pressure preclinical model of preeclampsia.
- Using a novel ratiometric mass spectrometry probe, we established that L-ergothioneine exerts its therapeutic effects, in part, by reducing the renal production of mitochondria-specific H_2O_2.

What Is Relevant?

- There is overwhelming evidence for deleterious role of exaggerated oxidative stress in the pathophysiology of preeclampsia, yet clinical trials of antioxidant interventions were not clinically effective in treating this disease.

- One potential explanation is that these antioxidants may have missed the intracellular mitochondrial reactive oxygen species production.
- This study provides further evidence that therapies targeting mitochondria are an exciting new avenue of investigation for the treatment of preeclampsia.

Summary

L-ergothioneine is a water-soluble amino acid with potent antioxidant properties. L-ergothioneine alleviates the clinical characteristics of preeclampsia, in part, via regulation of mitochondria-specific reactive oxygen species production in the reduced uterine perfusion pressure rat model of preeclampsia. Intriguingly, L-ergothioneine has been shown to have an encouraging safety profile in recent human studies, which further adds to its potential as a novel viable therapeutic for the prevention of preeclampsia.