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Attenuation of oxidative stress and hypertension in an animal model of HELLP syndrome

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ABSTRACT

HELLP (hemolysis elevated liver enzyme low platelet) syndrome is associated with hypertension, inflammation, oxidative stress and endothelial activation. The objective of this study was to determine if oxygen scavenging or endothelin A receptor antagonism improved hypertension and oxidative stress. sFlt-1 and sEndoglin were infused via mini-osmotic pump into normal pregnant rats (NP) on gestational day 12 to create HELLP syndrome. On gestational day 18 arterial catheters were inserted and on gestational day 19 mean arterial pressure was analyzed in rats; serum, urine and tissues were collected for molecular analysis. HELLP rats had significantly increased MAP compared to control normal pregnant rats ($P < 0.0005$). Endothelin A receptor antagonism via ABT-627 and Tempol, superoxide dismutase mimetic, were administered to a subset of normal pregnant and HELLP rats beginning on gestational day 13 and attenuated mean arterial pressure in HELLP rats ($P < 0.05$; $P < 0.005$). There were no statistically significant differences in mean arterial pressure between NP + ET_A Receptor or NP + Tempol treated rats and NP rats ($P = 0.22$). Endothelin A receptor blockade significantly decreased HELLP induced isoprostane excretion ($P < 0.0005$), placental and hepatic reactive oxygen species ($P < 0.05$; $P < 0.0005$) and increased placental total antioxidant capacity ($P < 0.005$) compared to untreated HELLP rats. Similar results in isoprostane ($P < 0.005$), hepatic reactive oxygen species ($P < 0.05$) and placental total antioxidant capacity ($P < 0.05$) were seen in HELLP rats treated with Tempol or Endothelin A receptor antagonist vs. untreated HELLP rats. These data demonstrated a role for oxidative stress in contributing to the hypertension, placental and liver damage that is seen in HELLP syndrome.

1. Introduction

Hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome is a severe complication of pregnancy that affects approximately 10–20% of women with preeclampsia (Abildgaard and Heimdal, 2013; Sibai, 2004; Weinstein, 1982). The mechanisms responsible for the pathogenesis and pathophysiology of HELLP syndrome remain unclear, however similar to preeclampsia inadequate invasion of the uterine spiral arteries is believed to be paramount to the development of this syndrome. Studies by our lab and others have previously reported that infusion or overexpression of two placental derived anti-angiogenic factors, soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin into pregnant rats produces hemolysis, increases liver enzymes and lower platelets in pregnant rats, leading to an animal model with HELLP-like characteristics (Venkatesha et al., 2006; Wallace et al., 2014b). In addition to the increase in hemolysis, liver enzymes, decrease in platelets, and hypertension that is present in this animal model during pregnancy, these rats also have increased circulating inflammatory cytokines, T lymphocytes and evidence of endothelial dysfunction that is also reported in women with HELLP syndrome (Abildgaard and Heimdal, 2013; Morris et al., 2016; Wallace et al., 2014b).

We have previously reported that endothelial cells exposed to media from placental ischemic rats have significantly more oxidative stress compared to endothelial cells exposed to media from normal pregnant rats (Wallace et al., 2014a), suggesting a relationship between these systems. Blockade of the endothelin A receptor has been shown to contribute to a decrease in hypertension and inflammation in animal

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models of preeclampsia as well as HELLP (Alexander et al., 2001; Morris et al., 2016). Similar results have been reported when oxidative stress is targeted in pregnant animal models of placental ischemia (Sedeek et al., 2008; Tam Tam et al., 2011; Wallace et al., 2014a); indicating a potential role for these systems in the hypertension, inflammation and syndrome severity. We have previously published data demonstrating infusion of the anti-angiogenic factors sFlt-1 and soluble endoglin into normal pregnant rats induces HELLP syndrome and induces hypertension and endothelin dysregulation (Morris et al., 2016; Wallace et al., 2014b). Our objective was to evaluate whether oxidative stress is increased in this animal model of HELLP and if either oxygen scavenging or endothelin A receptor antagonism improved the hypertension and oxidative stress.

2. Materials and methods

All studies were performed in 230–250 g timed-pregnant Sprague Dawley rats (Harlan). Animals were housed in a temperature controlled room with a 12:12 light:dark cycle. All experimental procedures in this study were in accordance with the National Institute of Health guidelines for use and care of animals and were approved by the Institutional Animal Care and Use Committee at The University of Mississippi Medical Center.

2.1. Animal model of HELLP syndrome

On gestational day (GD) 12, sFlt-1 and soluble endoglin (R&D systems) were infused at a rate of 4.7 and 7.0 μg/kg respectively via mini-osmotic pumps into normal pregnant (NP) rats to induce HELLP syndrome as previously described (Morris et al., 2016; Wallace et al., 2014b), normal pregnant rats not infused with sFlt-1 and soluble endoglin served as controls. On gestational day 18 carotid catheters were inserted into all rats and on gestational day 19 mean arterial pressure was measured as previously described (Amaral et al., 2014; Wallace et al., 2014a, 2014b, 2012, 2011), after which plasma, serum, urine and maternal tissues (placentas and liver) were immediately weighed and frozen at − 80 °C for future analysis. The biochemical components of HELLP syndrome were measured in plasma and whole blood: hemolysis (lactate dehydrogenase), elevated liver enzyme (aspartate aminotransferase) and platelet levels (Morris et al., 2016; Wallace et al., 2014b).

2.2. Administration of ABT-627 and Tempol

Beginning on gestational day 13 a separate set of normal pregnant and HELLP rats were treated with the endothelin A receptor antagonist ABT-627 (ET_A Receptor; 5 mg/kg, Abbott Laboratories, Abbott Park, IL) or with a superoxide dismutase mimetic, Tempol, (TEM; 5 mg/kg, Sigma, St. Louis, MO) in their drinking water until euthanization at GD19 resulting in NP (n = 7), NP + ET_A Receptor (n = 4), NP + TEM (n = 4), HELLP (n = 7), HELLP + ET_A Receptor (n = 7), HELLP + TEM (n = 8) groups to be studied.

2.3. Determination of oxidative stress

2.3.1. Determination of urinary isoprostane

On gestational day 19 urine was collected from the bladder and utilized for determination of excreted isoprostane (8-Iso-$PGF_2\alpha$) measured via ELISA from Oxford Biomedical Research (Oxford, MI). The assay displayed a sensitivity of 0.05 ng/ml, inter-assay variability of 4.2%, and intra-assay variability of 4.7%.

2.3.2. Placental nitrotyrosine activity

One placenta per rat was homogenized in radioimmunoassay precipitation buffer containing a protease inhibitor cocktail (Santa Cruz Biotechnology, Dallas, TX) followed and centrifuged at 500 × g for 10 min at 4 °C. The supernatant was assayed using a commercially available Nitrotyrosine ELISA from EMD Millipore (Darmstad, Germany). The assay was completed by following manufacturer's instructions which had a displayed sensitivity of 0.1 μM.

2.3.3. Reactive oxygen species detection

Placenta and livers (1:8 wet weight/volume) were homogenized in radioimmunoassay precipitation buffer containing a protease inhibitor cocktail (Santa Cruz Biotechnology) followed by centrifugation for 30 min at 500 × g at 4 °C, the supernatant was collected and the remaining cellular debris discarded. Briefly, 50 μl of the supernatant and 50 μl of Krebs-HEPES buffer (118 mM NaCl, 25 mM NaH_2CO_3, 48 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 11 mM Glucose, 1.5 mM $CaCl_2$, 25 mM HEPES) was added to a 96-well plate. Half of the wells (6 per sample) received 50 μl of 5 mg NADPH (beta-nicotinamide-adenine-dinucleotide 2' phosphate reduced tetrasodium salt; Sigma) while the remaining 6 wells received an additional 50 μl of Krebs-HEPES buffer to measure basal reactive oxygen species production. After all wells received lucigenin (final concentration 5 μM/L) samples incubated for 30 min in the dark before luminescence was measured via a plate reader (BioTek). Luminescence was recorded as relative light units (RLU) per min. An assay blank with no homogenate but containing lucigenin was subtracted from the reading before transformation of the data. The average of each sample was used for data transformation. The protein concentration was measured using a protein assay with BSA standards (Pierce, Rockford, IL). The data are expressed as RLU/min/mg protein.

2.3.4. Total antioxidant capacity

To determine the total antioxidant capacity of placenta and liver tissue, supernatant from tissue homogenates were assayed according to the manufacturer's directions (Cell Biolabs). The assay displayed sensitivity of 0.004 mM uric acid, inter-assay variability of 7.5% and intra-assay variability of 4%.

2.4. Statistical analysis

All data are expressed as S.E.M. The difference between the groups was determined by one-way analysis of variance (ANOVA) with Tukey's test of multiple comparisons as a post-hoc comparison. Data was considered statistically significant when $P < 0.05$.

3. Results

3.1. Oxygen Scavenging and blockade of the endothelin A receptor reduces mean arterial pressure in HELLP rats

Infusion of sFlt-1 and soluble endoglin into gestational day 12 normal pregnant rats significantly increases mean arterial pressure compared to control normal pregnant rats ($P < 0.0005$; Fig. 1). To determine if superoxide mimetic or blockade of the endothelin A receptor decreased hypertension and oxidative stress in pregnant rats with HELLP syndrome, Tempol and the endothelin A receptor were blocked beginning on gestational day 13. Administration of Tempol significantly decreased mean arterial pressure in HELLP rats ($P < 0.005$) as did blockade of the endothelin A receptor in HELLP rats ($P < 0.05$) compared to untreated HELLP rats (Fig. 1). There were no significant statistical differences ($P = 0.22$) in the mean arterial pressures between NP + TEM (111.7 ± 1.8 mmHg), NP + ET_A (100.5 ± 9.3 mmHg) or NP (97.3 ± 2.1 mmHg) rats when they were compared via one-way ANOVA. As such further analysis did not include NP + TEM or NP + ET_A.

There were no statistically significant differences between the groups in pup weight ($P = 0.59$), placental weight ($P = 0.82$), kidney weight ($P = 0.39$) or in liver weight ($P = 0.13$). Treatment with the endothelin A receptor antagonist significantly reduced spleen weight (0.64 ± 0.05; $P < 0.005$) relative to untreated HELLP rats.