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Abstract—Recently we described a mouse model, BPH/5, that spontaneously develops the hallmark clinical features of preeclampsia. BPH/5 exhibit impaired placentation before the onset of hypertension and proteinuria, supporting a causal role for the placenta in the pathogenesis of preeclampsia. Here we tested the hypothesis that an increase in reactive oxygen species (ROS) early in pregnancy results in placental abnormalities leading to the maternal symptoms of preeclampsia. We further hypothesized that chronic antioxidant therapy would ameliorate both feto-placental abnormalities and maternal symptoms. ROS levels measured by dihydroethidium revealed significant increases in oxidative stress in BPH/5 placentas at midgestation compared with C57 controls. This increase in ROS was correlated with reduced expression and activity of cytoplasmic superoxide dismutase in early and midgestation BPH/5 placentas. These abnormalities in placental oxidant factors occurred before the onset of maternal symptoms, suggesting a possible causal link between increased ROS and maternal and feto-placental pathology in this model. In support of this, chronic treatment of BPH/5 with the superoxide dismutase-mimetic Tempol throughout gestation significantly improved fetal growth and survival. Furthermore, Tempol ameliorated pregnancy-induced increases in blood pressure and proteinuria in BPH/5 mothers. We confirmed that Tempol radical was present in plasma, and it normalized ROS levels in all placental zones in BPH/5. These data for the first time demonstrate an important causative role for increased ROS in the placenta in the pathogenesis of preeclampsia in a model that spontaneously develops the disease. The results also strongly suggest the potential utility of antioxidant therapy in treating preeclampsia. (*Hypertension*. 2008;51:1058-1065.)

Key Words: antioxidants ■ oxidative stress ■ placenta ■ pregnancy-induced hypertension ■ preeclampsia

Although the etiology of preeclampsia is unknown, the placenta is strongly implicated in the development of maternal symptoms because removal of the placenta results in rapid amelioration of maternal symptoms. The preeclamptic placenta is often poorly perfused, because of maladaptation of the spiral arteries supplying maternal blood to the placental bed.[1] These findings have led to a commonly-held notion that impaired placental perfusion early in gestation and subsequent ischemia results in the release of factors from the placenta into the maternal circulation, which initiate the maternal disease cascade.[2] However, the precise identities of the released factors are not known. More importantly, the placental molecular events leading to the release of these factors are not well-established.

Several lines of research have suggested a role for increased oxidative stress in preeclampsia. For example, both reduced expression of antioxidant enzymes and increased markers of oxidative stress including increased protein carbonyls and lipid peroxidation have been reported both in term human placentas as well as maternal leukocytes, vasculature, and plasma.[3–5] In addition, increasing antioxidant capacity by vitamins C and E supplementation during pregnancy have had some beneficial effects in prevention of preeclampsia in women with increased risk of developing the disease.[6] However, the early gestation events that lead to development of oxidative stress are not known. It has been suggested that ischemia in the developing placenta likely results in an increase in production of reactive oxygen species (ROS), and if coupled with deficient antioxidant enzyme expression, could result in a state of heightened oxidative stress.[7]

Changes in ROS levels and antioxidant enzymes in the preeclamptic placenta during early pregnancy have remained largely unknown, in part because of the difficulty of prediction of at-risk individuals before they develop the disease, challenges in acquiring placental samples in early pregnancy, and the lack of animal models that spontaneously develop the

disease and would allow ROS analysis and testing of therapeutic strategies at early time-points before term. Recently, we described a mouse model of preeclampsia (BPH/5) that develops the cardinal features of the disease.[8] In addition to developing late gestational hypertension and proteinuria, BPH/5 mice also exhibit poor placental development before the onset of maternal symptoms, supporting a causative role for the placenta in this model.[9]

Using this model, we tested the hypothesis that an early increase in placental oxidative stress, before the onset of maternal symptoms, plays a central role in placental dysfunction and maternal disease, and that antioxidant treatment would interrupt the disease process. We tested this hypothesis by evaluating placental ROS levels, antioxidant enzyme expression, and by using chronic antioxidant therapy using the superoxide dismutase (SOD) mimetic Tempol throughout gestation. Our results demonstrate a marked increase in ROS levels in the placenta at midgestation attributable to a decrease in the activity and levels of cytoplasmic SOD (CuZnSOD). Furthermore, scavenging of ROS with Tempol was effective in ameliorating the onset of feto-placental abnormalities and maternal symptoms. These data suggest a causative role for placental oxidative stress in the development of preeclampsia in this model.

Materials and Methods

Animals and Husbandry

Animals used in this study were cared for and bred as described previously.[8] Briefly, 2- to 4-month-old BPH/5 and C57 female mice were mated with strain-matched males and day of plug detection was designated as day 0.5 of pregnancy. Mice were either given normal drinking water or water supplemented with 1mmol/L Tempol (4-hydroxy-2,2,6,6-tetramethyl-piperidinoxyl, Sigma), and water bottles were changed weekly. Tempol treatment was started 2 days before mating. One group of mice was implanted with radiotelemeters for blood pressure analysis (see below) and another cohort was euthanized at 3 time points during pregnancy representing early and midgestation (days 12.5 and 14.5) for collection of placental samples. To confirm that water intake and body weight was not altered by treatment with Tempol in the drinking water, daily water intake was measured and mice were weighed weekly in a subset of BPH/5 mice. All animal experimental protocols in this study were approved by the University of Iowa Animal Care and Use Committee. Care of the animals met or exceeded the standards set forth by the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health.

Western Blot Analysis and SOD Activity Assay

Three placentas pooled from each litter collected at early and midgestation were homogenized in 50 mmol/L phosphate buffer (pH 7.8) for Western and enzyme activity analyses. For Western blotting, equal amounts of proteins from different groups ($\approx$30 μg) were electrophoresed in a 12.5% SDS-PAGE gel, and proteins were transferred to nitrocellulose membranes and blocked in 5% dry milk in TBST (0.01 mol/L Tris/0.15 mol/L NaCl buffer, pH 8.0 and 0.1% Tween 20) at room temperature for 1 to 2 hours. CuZnSOD levels were measured as described previously using a polyclonal rabbit anti-CuZnSOD antibody (Upstate Cell Signaling Solutions).[10] Band intensities were normalized to actin. These corrected values were normalized to the day 12.5 C57 band for each blot, to correct for differences in exposure length in different blots. Experiments were repeated with 3 separate BPH/5 and C57 groups.

SOD activity was measured in placental homogenates by quantifying inhibition of xanthine-xanthine oxidase–mediated reduction of Nitroblue Tetrazolium (NBT) as described.[11] CuZnSOD activity was determined by measuring the NaCN-inhibitable fraction of total SOD activity. One unit of SOD activity is defined as the amount of protein required to obtain half-maximal inhibition of NBT reduction.

Dihydroethidium Staining in Placental Sections

Pregnant mice treated with or without Tempol treatment were euthanized at midgestation and placentas were immediately removed, frozen, and cryosectioned at 10 μm. Detection of superoxide levels using dihydroethidium (DHE) was performed as described previously.[12,13] Sections were incubated with 1 μmol/L DHE (Molecular Probes) in PBS for 15 minutes. After DHE loading, slides were washed with PBS, coverslipped, and fluorescence was analyzed and digital images were captured by confocal microscopy (Zeiss, LSM 510). Laser settings were kept constant for all sections in each group to enable direct comparisons. Fluorescence intensity was quantified using ImageJ software as described previously.[13]

Radiotelemetric Measurement of Blood Pressure

Longitudinal measurement of blood pressure was carried out as described previously.[8,14] Briefly, a cohort of mice were implanted with PA-C20 or PA-C10 radiotelemeters (Data Sciences International) and allowed to recover for 5 days before baseline measurements were taken for 2 days. Treatment with Tempol (1mmol/L) or normal water was started and 2 days later, males were placed in the cages for mating. After plug detection, blood pressure was recorded as described previously.[8]

Assessment of Urinary Protein

Measurements of protein levels in urine samples of nonpregnant and pregnant C57 and BPH/5 females with or without Tempol treatment were performed using Bradford reagent (Biorad, Hercules, Calif) as previously described.[8]

Electron Spin Resonance Detection of Tempol

Electron spin resonance (ESR) was performed in plasma and urine from pregnant C57 and BPH/5 mice in the ESR facility at the University of Iowa (Bruker EMX ESR Spectrophotometer with a TM cavity and an aqueous flat cell).[15] Briefly, samples stored at $-80°$C were thawed and diluted in air-saturated PBS to ensure correct sample volume. ESR spectra were acquired and peak heights were quantified and compared against Tempol standard solutions in PBS to determine absolute levels of Tempol. Instrument settings for all samples were: sweep rate 60 G/84 s for 8 scans; nominal power, 80 mW; modulation amplitude 1 G; time constant, 82 ms. At the end of the experiment, addition of potassium ferrocyanide or ascorbate oxidase confirmed that all Tempol was present in the ESR-detectable nitroxide form.[16]

Statistical Analyses

Data are expressed as mean$\pm$SEM. In experiments comparing only untreated BPH/5 and C57, data were analyzed using Student t test. In experiments where treated and untreated groups of both strains were compared, 1-way ANOVA was used with the Newman-Keuls post-hoc tests to determine significance. $P<0.05$ was considered statistically significant.

Results

CuZnSOD Protein Levels and Activity Are Reduced in BPH/5 Placentas Compared With C57 Controls

During early gestation, successful transition from hypoxia to normoxia in the placenta requires extensive angiogenesis and remodeling of the maternal arteries to meet the demands of the growing feto-placental unit. It has been suggested that this transition also causes increased free radical production in the placenta attributable to upregulation of superoxide generating enzymes[17] but this is counterbalanced by upregulation of
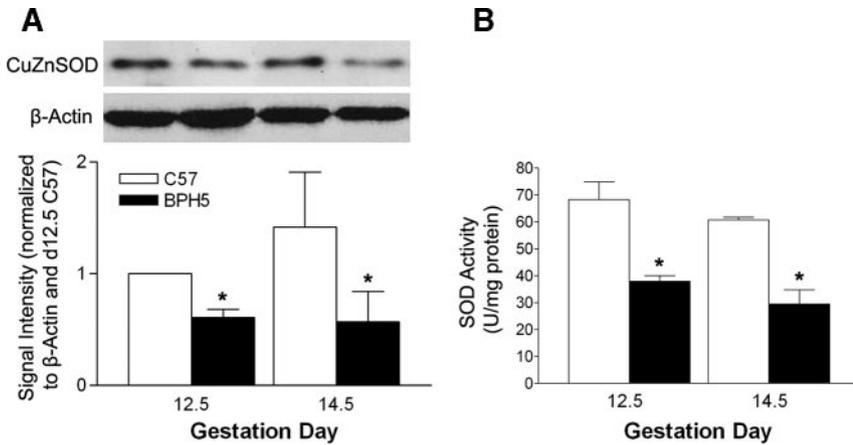
Figure 1. BPH/5 placenta exhibits decreased levels of the antioxidant enzyme CuZnSOD expression and activity. A, Representative Western blot and summary data (n=3) showing decreased CuZnSOD protein levels in BPH/5 placenta at early- and midgestation time points. B, Spectrophotometric assay of CuZnSOD activity measurements confirming reduced CuZnSOD activity at early- and midgestation time points (n=3). *$P<0.05$ BPH/5 vs C57.

antioxidant enzymes in normal pregnancy.[17,18] Failure to adequately upregulate these antioxidant enzymes in placenta could lead to marked and sustained oxidative stress. Based on these findings, we hypothesized that a defect in upregulation of superoxide scavenging enzymes in BPH/5 placentas precedes the development of maternal symptoms. To test this, we measured CuZnSOD and MnSOD protein levels and enzyme activity in BPH/5 placentas at early and mid gestation. Western analysis revealed a significant decrease in CuZnSOD protein levels in placental homogenates from BPH/5 as compared with C57 at both early and mid-gestation (Figure 1A). Spectrophotometric assay for SOD activity confirmed that reductions in protein levels were associated with a marked decrease in CuZnSOD activity in BPH/5 placentas at both gestational stages compared with gestation age-matched C57 controls (Figure 1B). Westerns and activity assays for the mitochondrial SOD isoform, MnSOD, revealed no significant changes at either gestational stage (data not shown).

Superoxide Levels Are Increased in BPH/5 Placentas Compared With C57 Controls

Based on the discovery that CuZnSOD expression was blunted early in pregnancy, we hypothesized that BPH/5 placentas would show increased levels of superoxide. We tested this hypothesis by measuring superoxide levels in different layers of midgestation BPH/5 placentas using a superoxide-sensitive fluorescent probe, dihydoethidium (DHE). As shown in the representative DHE fluorescence images in Figure 2A, superoxide levels are markedly increased in each of the major placental zones, including labyrinth, junctional, and decidual layers of BPH/5 placentas as compared with age-matched C57 control placentas. Sum-

mary data from 5 to 8 individual placentas from each group presented in Figure 2B show that superoxide levels in BPH/5 are significantly elevated compared with controls. Together, these data suggest that BPH/5 placentas develop marked and sustained oxidative stress before late-gestational preeclampsia symptoms, likely in part because of a defect in induction of CuZnSOD expression.

Chronic Tempol Treatment Prevents Development of Hypertension and Proteinuria in BPH/5 Mice

Based on our findings of decreased CuZnSOD expression with a concomitant increase in oxidative stress in BPH/5 placentas we hypothesized that chronic treatment of BPH/5 mice throughout gestation with the SOD mimetic Tempol may ameliorate both maternal and fetal disease. To test this hypothesis we treated BPH/5 and control C57 mice with Tempol (in the drinking water) starting 2 days before pregnancy and compared the blood pressure profile of Tempol-treated and untreated (normal drinking water) BPH/5 and C57 mice throughout gestation. Data presented in Figure 3A show that Tempol treatment had no significant effect on baseline blood pressure before, during, or after pregnancy in C57 mice. However, as seen in Figure 3B, the late-gestational rise in blood pressure that is characteristic of BPH/5 pregnancy,[8] was prevented by chronic treatment with Tempol. Similar to its effects on hypertension, chronic Tempol treatment prevented the characteristic development of late-gestational proteinuria in BPH/5[8] (Figure 3C). Again, Tempol did not alter urinary protein levels in C57 mice at any gestational age. To ensure that chronic Tempol had no effect on body mass or water intake, in separate subsets of nonpregnant BPH/5 and C57 mice we measured these end points daily for 2 weeks. As shown in the Table for BPH/5 mice, Tempol did not alter
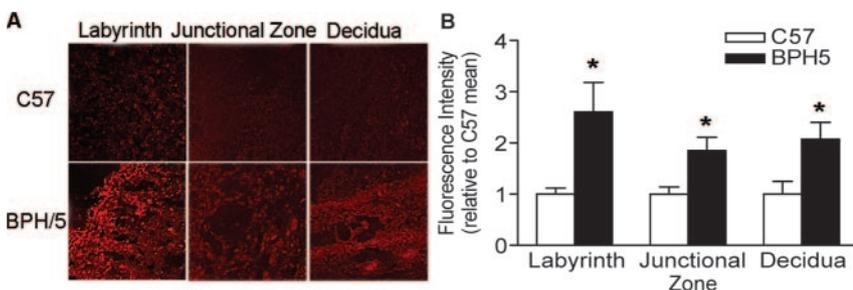

Figure 2. BPH/5 placentas exhibit increased superoxide levels. A, Representative confocal images of DHE stained sections from mid-gestation BPH/5 and C57 placentas. B, Summary data showing a significant increase in superoxide levels in all 3 placental zones in BPH/5 relative to time-matched C57 controls. (n=5 to 8 placental sections per group). *$P<0.05$ BPH/5 vs C57.
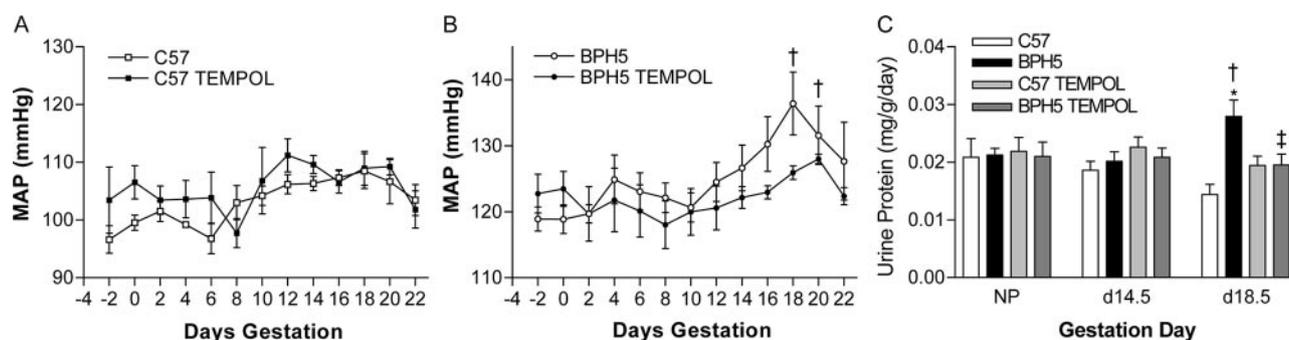
Figure 3. Maternal symptoms of preeclampsia are prevented by treatment with Tempol. Summary data of longitudinal measurements of blood pressures throughout pregnancy in C57 (A) and BPH/5 (B) mice treated with or without Tempol (n=6 to 9 in each group). Tempol treatment of BPH/5 mice completely abolished the pregnancy-induced increase in blood pressure with no significant effect in C57 mice. C, Summary data showing Tempol treatment-dependent decrease in late gestational protein secretion in urine in BPH/5 mice (n=6 to 15 in each group). *$P<0.05$ BPH/5 vs C57, ‡$P<0.05$ BPH/5 vs BPH/5+Tempol, †$P<0.05$ vs baseline (day 0.5).

weight gain or amount of water consumed daily compared with mice drinking normal water. Similar results were found for C57 (data not shown). These data suggest a causative role for increased ROS in pregnancy-induced hypertension and proteinuria. Taken together with data showing dysregulation of ROS-scavenging enzymes in the placenta, these results indicate a possible link between oxidant stress in the placenta and the maternal preeclampsia syndrome.

Feto-Placental Outcomes in BPH/5 Mice Are Normalized by Treatment With Tempol

We have previously shown that feto-placental development is impaired in the BPH/5 model before the onset of hypertension and proteinuria.[8,9] Therefore, to determine whether increased placental oxidative stress is an important contributor to feto-placental abnormalities observed in this model, we compared feto-placental outcomes in the BPH/5 and C57 mice with or without Tempol treatment. The progressive fetal demise seen over gestation in the BPH/5 strain[8] was significantly attenuated by Tempol as seen by a reduction in the incidence of fetal reabsorption by more than 50% as compared with untreated BPH/5 (Figure 4A). This translated into a significant increase in term litter size in Tempol-treated BPH/5 as compared with untreated BPH/5 (Figure 4B). In addition to enhancing survival, the feto-placental units in Tempol-treated BPH/5 were more healthy, exhibiting normalized placental weights at midgestation (Figure 4C) and restored fetal weights in late pregnancy (Figure 4D). These outcomes were not associated with any teratogenic effects in either strain, and pups born from Tempol-treated mothers developed normally after birth (data not shown). It should be noted that Tempol had no significant effect on any of these

outcomes in C57. Together, these results suggest that normalizing ROS levels in BPH/5 diminishes feto-placental growth impairment and fetal mortality, and implicates oxidant stress in these preeclampsia-associated outcomes.

Chronic Tempol Treatment Normalizes Superoxide Levels in BPH/5 Placentas

To demonstrate that Tempol treatment is improving feto-placental outcomes and maternal symptoms at least in part by reducing placental oxidative stress, we compared ROS levels in mid-gestation placentas from Tempol-treated and untreated C57 and BPH/5 mice. As shown in representative images in Figure 5A and summary data presented in Figure 5B, all zones of the BPH/5 placenta showed effective scavenging of superoxide by Tempol such that the level of DHE fluorescence in Tempol-treated BPH/5 placentas was similar to levels seen in C57 with or without Tempol treatment. These findings demonstrate that orally-administered Tempol circulates at robust levels early in pregnancy and is able to efficiently scavenge excess ROS levels in placenta.

Plasma Tempol Levels Were Comparable in C57 and BPH/5 Mice Receiving Oral Tempol

Finally, to analyze Tempol levels in vivo and to confirm that there are no strain-related differences in metabolism of the drug, plasma, and urinary concentrations of the Tempol radical were evaluated by electron spin resonance (ESR) spectrophotometry. Representative ESR tracings in Figure 6A show the 3 characteristic Tempol spectral signals[19] in plasma samples from Tempol-treated C57 and BPH/5 mice (no signals were detected in any samples from non-treated mice, data not shown). Analysis of the Tempol-associated spectral peak heights revealed the presence of significant amounts of Tempol in the plasma that were similar in C57 and BPH/5 (6 to 7 μmol/L) (Figure 5B). Not surprisingly, high levels of Tempol radical were found in maternal urine of both C57 and BPH/5 mice receiving Tempol, and these also were not different between the 2 strains. (Figure 5B).

Discussion

In this study, using the BPH/5 mouse model of preeclampsia, we investigated the role of ROS in the pathophysiology of

Table. Water Intake and Body Weight Gain During Tempol Treatment in BPH/5

Treatment	Weight Gain, g		Average Daily Water Intake, mL	
	Week 1	Week 2	Week 1	Week 2
Control water	1.54±0.20	−0.13±0.11	3.73±0.26	3.60±0.25
Tempol water	1.52±0.33	−0.59±0.28	3.92±0.21	3.74±0.26

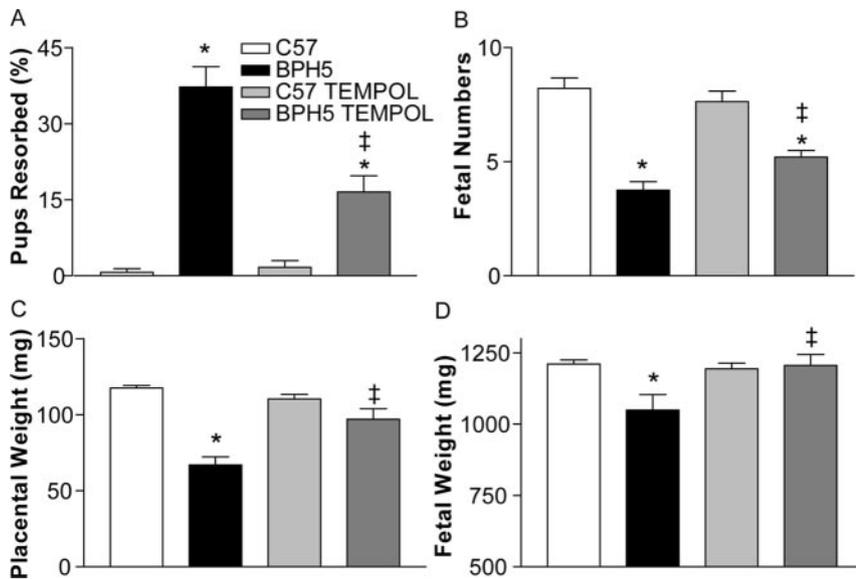Results are mean±SEM (n=15, both groups).

Figure 4. Tempol treatment significantly reduces early fetal demise and normalizes placental weights and term fetal weights. A, Incidence of reabsorption of BPH/5 placentas (% of the litter resorbed) was significantly attenuated by treatment with Tempol (n=13 to 27 per group). B, Prevention of early fetal demise by Tempol resulted in significantly larger litters in BPH/5 (n=14 to 29 per group). C, Midgestational Placental mass in Tempol-treated BPH/5 was restored to C57 levels (n=20 to 57 per group). D, Tempol treatment of BPH/5 mice also increased late-gestational fetal weights which were similar to C57 fetal weights (n=25 to 60 per group). *$P<0.05$ BPH/5 vs C57, ‡$P<0.05$ BPH/5 vs BPH/5+Tempol.

this syndrome by systematically analyzing placental antioxidant enzymes and levels of superoxide throughout pregnancy, as well as using an antioxidant treatment strategy to causally link ROS to the development of late-gestational hypertension, proteinuria, and poor feto-placental outcomes. Here, we present evidence that supports the hypothesis that elevated ROS levels in BPH/5 placental plays a key role in the development of both feto-placental abnormalities and maternal disease symptoms. The following evidence supports this hypothesis: (1) early gestation BPH/5 placentas fail to show induction of CuZnSOD to the level of C57 controls; this coincided with a marked increase in ROS levels in BPH/5 placentas as compared with C57; (2) chronic treatment of BPH/5 mice with the SOD mimetic Tempol improved feto-placental outcomes and abolished late-gestational hypertension and proteinuria; (3) Tempol was detected at significant levels in the plasma of Tempol-treated mice and Tempol treatment restored placental superoxide to C57 levels. Collectively, these studies suggest a strong link between placental oxidative stress and the pathogenesis of preeclampsia, and highlight the potential value of antioxidant treatment in preventing the disease.

Establishment of blood flow to the placenta during early gestation results in a burst of superoxide generation by the activation of xanthine oxidase[20] and NADPH oxidase.[21,22] In normal pregnancy this is balanced by induction of antioxidant enzymes.[20] An imbalance in these pro- and antioxidant forces has been proposed to lead to increased placental oxidative stress and the development of preeclampsia.[3,4] Our findings of decreased levels of CuZnSOD in BPH/5 placentas before development of maternal hypertension and proteinuria support this concept and implicate defective superoxide scavenging as a source of oxidant stress in this model. In fact, decreased SOD levels have been observed in term placental samples from preeclamptic women.[3,4] Other studies have suggested there may be dysregulation of several other placental antioxidant systems including catalase, GPx, and others.[3,4] Interestingly, no studies of CuZnSOD mutant transgenic mice to date indicate developmental or reproductive abnormalities. This suggests that the antioxidant profile of the placenta is diverse and involves multiple overlapping antioxidant pathways. This also suggests that excess production of ROS plays just as significant a role as deficient scavenging in
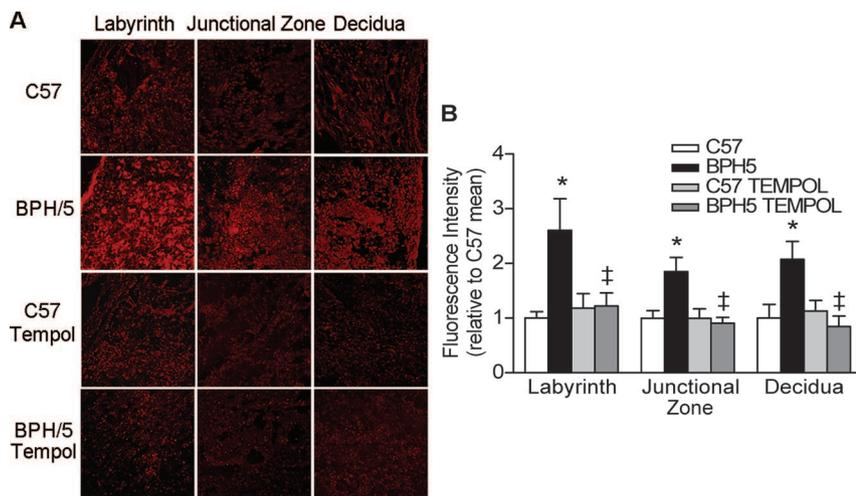

Figure 5. Increased superoxide levels in the BPH/5 placenta are restored to normal by Tempol treatment. A, Representative confocal images of DHE-stained placental labyrinth, trophoblast, and decidua zones from midgestation placental sections of BPH/5 and C57 mice treated with or without Tempol. B, Summary of DHE fluorescence quantification demonstrating elevated ROS levels in all 3 zones of mid-gestational BPH/5 placenta that is normalized to near C57 levels by treatment with Tempol. (n=5 to 8 placental sections per group). *$P<0.05$ BPH/5 vs C57, ‡$P<0.05$ BPH/5 vs BPH/5+Tempol.
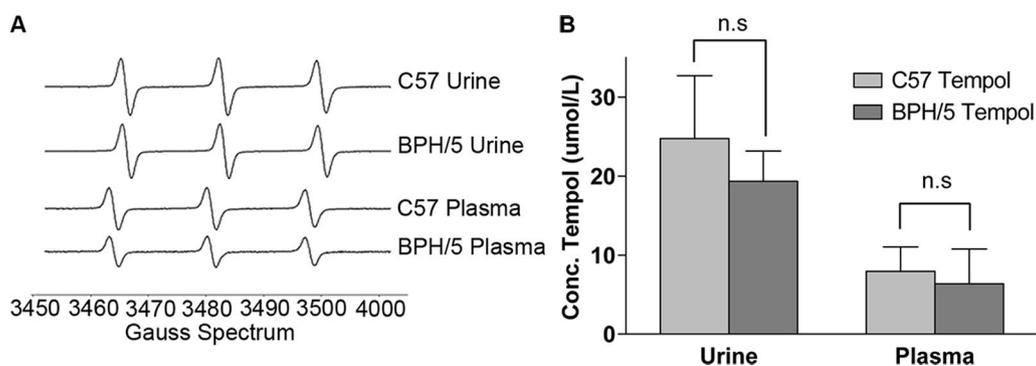
Figure 6. Tempol radical is present at comparable levels in plasma and urine of Tempol-treated C57 and BPH/5 mice. A, Representative Tempol radical EPR spectra from plasma samples of C57 and BPH/5 treated with or without Tempol. Three peaks are associated with the Tempol radical. B, Summary data demonstrating significant and comparable levels of Tempol radical in maternal plasma and urine of C57 and BPH/5 mice (n=3 to 4).

the generation of oxidative stress in early pregnancy. Although the mechanisms underlying altered expression of oxidant and antioxidant enzymes in preeclampsia are not known, emerging evidence has implicated the renin-angiotensin system in the early gestational activation of the NAD(P)H oxidase in preeclamptic placenta.[23] Additionally, intermittent ischemia/reperfusion caused by inadequate trophoblast remodeling of decidual arteries during establishment of placental blood flow has been suggested to play an important role in the activation of xanthine oxidase.[4] Because steady-state ROS levels in the placenta are the net result of superoxide generation and the level of antioxidant enzymes expressed in placenta, in future studies it will be important to investigate the expression and activation of these prooxidant enzymes in placentas of BPH/5 mice with the goal of understanding the mechanisms of elevated placental ROS production in early pregnancy.

Similarities between BPH/5 placentas and term placentas obtained from human preeclamptic patients with regard to oxidant factors[3,4] not only demonstrate the suitability of the BPH/5 mouse model to investigate the mechanisms of increased ROS production but also provide an appropriate animal model to address the question raised by Roberts and Hubel: "is oxidative stress the link in the two-stage model of preeclampsia[24]?" The nature of the two-stage disease model implies that defects in placental development during early gestation result in dysfunction of the maternal vasculature, leading to development of preeclampsia. Evidence presented here and clinical studies strongly implicate placental ROS dysregulation in the development of preeclampsia.[3,4] However, there is also abundant evidence that oxidative stress plays an important role in the maternal vasculature in preeclampsia by way of decreased expression of antioxidant enzymes and increased markers of oxidative stress in blood vessels,[3–5] changes in vascular function,[25] and increased placental production of factors known to induce ROS production (ie, tumor necrosis factor [TNF]-α, angiotensin type 1 receptor autoantibodies).[23,26] Indeed, although our studies strongly implicate the importance of the placenta, we cannot rule out the possibility that these or other factors are not playing a role, and that Tempol is in part having its beneficial effects by scavenging ROS in other tissues. In addition, whether these maternal changes are primary or secondary to

placental oxidative stress in preeclampsia is an important area of investigation and use of animal models like the BPH/5 mouse and the reduced uterine perfusion pressure (RUPP) rat model[27] is expected to advance our understanding of fetoplacental-maternal interaction in the progression of the disease. Recent work suggests another possible link between placental dysfunction and maternal vascular disease—production and balance of pro- and antiangiogenic factors.[28,29] ROS have been established as key players in mediating angiogenesis,[30,31] so investigating the linkage between dysregulation of ROS and angiogenic balance in BPH/5 is an important goal for future study.

It should be noted that although the hypothesis that increased oxidative stress in placenta and maternal vasculature plays a key role in the development of preeclampsia is gaining wide support, the results of antioxidant therapy for treatment of preeclampsia have been disappointing so far. In contrast to an earlier small randomized trial showing beneficial effects of vitamin C and E supplementation during early gestation (16 to 22 weeks of pregnancy) in the prevention of preeclampsia in women at increased risk for the disease,[6,32] recent multicenter randomized placebo-controlled trials in large number of nulliparous women[33] as well as in women with increased risk of preeclampsia[34] have failed to show beneficial effects of combined vitamin C and E treatment in reducing the risk of preeclampsia. Recent meta-analysis of all eligible vitamin C and E supplementation trials in preeclampsia has concluded that "the available evidence does not support the combined vitamin C and E supplementation during pregnancy for the prevention of preeclampsia."[35] With the overwhelming evidence of increased oxidative stress during preeclampsia, the reasons for lack of beneficial effects of antioxidant vitamins are unclear. Possible explanations for these inconsistencies could be differences in study population and criteria for patient selection, etc. Moreover, the lack of mechanistic studies addressing the bioavailability and pharmacokinetics of these antioxidant vitamins and their ability to modulate oxidative stress in preeclamptic women makes it difficult to interpret the results of these clinical trials.

To overcome some of these limitations, we tested the effects of a different class of antioxidant, the SOD mimic Tempol, and started antioxidant therapy before the beginning of pregnancy in the BPH/5 model. In our studies, chronic

treatment with Tempol was able to prevent the development of hypertension and proteinuria during late gestation in BPH/5. These maternal changes were preceded by improvement in feto-placental growth and survival, suggesting a causative role for oxidative stress in mediating feto-placental dysfunction in this model. Most importantly, we were able to show significant levels of circulating Tempol and that treatment with this reagent efficiently normalized placental ROS levels in BPH/5 mice. It will be interesting to see whether combined therapy with vitamin C and E started before onset of pregnancy has similar effects to Tempol in this model. Tempol has been well-characterized as a superoxide scavenger, however several other related antioxidant functions have been described with respect to Tempol in in vitro systems, including termination of radical chain reactions, facilitating heme-mediated removal of H_2O_2, and reduction of hypervalent metals.[36,37] The DHE studies presented here indicate the importance of superoxide scavenging in this study, however we cannot rule out the involvement of these side reactions in the overall effects of Tempol treatment in BPH/5. Although Tempol is not being used in the clinical setting for treatment of cardiovascular disease, the drug is being evaluated clinically for treatment of hair loss with positive Phase I results.[38] Furthermore, studies in animals have demonstrated the effectiveness of this potent superoxide scavenger in improving cardiovascular outcomes in numerous experimental models of hypertension and cardiac and vascular dysfunction.[39–41]

Perspectives

Several studies have demonstrated strong association between oxidative stress in the placenta or maternal vasculature and the etiology of preeclampsia.[3,4] However, very little is known about the mechanisms leading to this oxidative stress. More importantly, a causative role for ROS in the pathophysiology of preeclampsia has been difficult to establish because of the lack of animal models that are suitable for prospectively testing the hypothesis starting early in gestation, and the obvious difficulty in studying early gestation placentas in women. Our results, demonstrating increased oxidative stress at least in part attributable to a defect in gestation-dependent upregulation of cytoplasmic SOD in the BPH/5 placenta, and chronic treatment with the SOD mimetic Tempol ameliorating both placental defects and maternal disease which is linked to normalization of placental oxidative stress suggest that BPH/5 mice will be an excellent animal model for such studies. We anticipate this model can be used to compare effectiveness of different antioxidants and combinations thereof for treatment of preeclampsia.

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