 Increased Myeloperoxidase in the Placenta and Circulation of Women With Preeclampsia


Abstract—Myeloperoxidase (MPO) is a hemoprotein normally released from activated monocytes and neutrophils. Traditionally viewed as a microbicidal enzyme, MPO also induces low-density lipoprotein oxidation, activates metalloproteinases, and oxidatively consumes endothelium-derived NO. The elevated plasma MPO level is a risk factor for myocardial events in patients with coronary artery disease. Patients with preeclampsia display evidence of the inflammation and endothelial dysfunction associated with oxidative stress in the circulation, vasculature, and placenta. We hypothesized that MPO levels in the circulation and placental extracts from women with preeclampsia would be greater than levels in women with normal pregnancies. Placental extracts were prepared from placent al villous biopsies from preeclamptic (n=27) and control (n=43) placentas. EDTA plasma samples were obtained from gestationally age-matched preeclamptic and control normal pregnancies. MPO concentrations were measured by ELISA. Immunohistochemistry was used to determine MPO localization in the placenta. MPO levels in placental extracts from women with preeclampsia were significantly higher than the levels in normal control subjects (546±62 versus 347±32 ng/mL; P=0.025). MPO was found in the floating villi and basal plate of placentas with a greater staining in the basal plates from preeclampsia placentas compared with normal pregnancies. Plasma MPO levels were 3-fold higher in patients with preeclampsia compared with normal control subjects (36.6±7.6 versus 11.0±3.1 ng/mL; P=0.003). In conclusion, MPO levels are significantly increased in the circulation and placenta of women with preeclampsia. We speculate that MPO may contribute to the oxidative damage reported in the endothelium and placenta of women with preeclampsia. (Hypertension. 2008;52:387-393.)

Key Words: preeclampsia ■ pregnancy ■ inflammation ■ peroxidase ■ placenta ■ chorionic villi ■ postpartum period

Normal human pregnancy is associated with maternal systemic inflammation that is exaggerated in preeclampsia, the pregnancy disorder characterized by hypertension developing after the 20th week of gestation and proteinuria. Preeclampsia is associated with increased neutrophil counts. Peripheral blood leukocytes are activated in women with normal third trimester pregnancies compared with nonpregnant patients. Leukocytes in normal pregnancy have increased markers of activation (CD11b, CD14, CD64, and intracellular reactive oxygen species), which are further increased in preeclampsia. Monocytes are also activated in pregnancy as evidenced by elevated surface expression of CD11b, CD14, and CD64 by the third trimester of pregnancy. Monocyte activation progressively increases and peaks at 29 to 36 weeks of gestation. There are several lines of evidence of neutrophil activation during preeclampsia, including the following: (1) greater basal and N-formyl-methionyl-phenylalanine (fMLP)-induced superoxide production; (2) increased CD11b expression basally in neutrophils from women with preeclampsia compared with normal pregnancies; and (3) increased neutrophil elastase.

Myeloperoxidase (MPO) is a hemoprotein normally produced and released by activated monocytes and neutrophils. It is traditionally recognized as a microbicidal enzyme primarily through the action of hypochlorous acid. MPO is associated with vascular dysfunction and is mechanistically linked to the pathophysiology of numerous vascular inflammatory diseases, including arteriosclerosis and coronary artery disease. Elevated circulating MPO levels are a risk factor of myocardial events in patients with coronary artery disease. There is a strong relationship between serum MPO levels and endothelial dysfunction in humans. Patient with cardiovascular disease (diagnosed as coronary artery disease, peripheral arterial disease, or cerebral vascular disease) and the
highest quartile of serum MPO were >6 times as likely to have endothelial dysfunction assessed by brachial artery flow-mediated dilation than patients in the lowest quartile.11

MPO has been shown to mediate oxidation of lipoproteins and to catalyze nitration of tyrosine residues and has been implicated in the depletion of endothelial-derived NO.12–16 Once in the circulation, MPO can become sequestered in the subendothelial space through a process of heparin glycosaminoglycan-dependent binding and endothelial transcytosis, resulting in its accumulation in the endothelial cell matrix.12 There it can be a potent source of reactive oxygen and nitrogen species and consume antioxidants.12,17,18 Activated MPO also catalyzes oxidation of nitrite and NO, ultimately causing nitration of protein tyrosines.12–15 Likewise, increased nitrotyrosine in blood vessels of the placenta and peripheral circulation of the mother19,20 is suggestive of MPO overactivity in the vasculature during preeclampsia. There are reports of increased or unchanged MPO in the circulation of women with preeclampsia.21 Women with a history of preeclampsia are at increased risk for poor cardiovascular health later in life.22 These observations prompted us to examine MPO levels in the placenta and circulation of women with preeclampsia.

Methods

Preeclampsia was defined using the criteria of gestational hypertension, proteinuria and hyperuricemia, and reversal of hypertension and proteinuria after delivery. Gestational hypertension was defined as systolic blood pressure >140 mm Hg systolic or diastolic blood pressure >90 mm Hg arising after 20 weeks’ gestation in a previously normotensive woman. Proteinuria was defined as >300 mg of protein in a 24-hour urine collection, >2+ on a voided or >1+ on a catheterized random urine sample, or a random urine protein:creatinine ratio of >0.3. Hyperuricemia was defined as >1 SD above normal for the given gestational age (at term: >5.5 mg/dL [3.3 mmol/L]). Control groups were composed of women with uncomplicated, normotensive pregnancies who delivered healthy, non-small-for-gestational-age babies. Patients with multiple gestations, chronic hypertension, diabetes, premature rupture of membranes, renal disease or other significant metabolic disorder, or a history of illicit drug use were excluded. The institutional review board approved the study, and written informed consent was obtained. Procedures followed were in accordance with institutional guidelines.

Placental biopsies were obtained immediately after cesarean delivery from a site between the placental rim and cord insertion. Clinical data for these patients are provided in Table 1. The tissue was quickly washed 3 times in physiological saline, frozen in liquid nitrogen, and stored at -70°C until use. Placental extracts were prepared from ~100-mg wet weight placental biopsy samples. Total proteins from placental tissues were extracted by homogenizing by sonication (Ultrasonic Processor, Tekmar, microprobe setting 70 for 30 seconds) in 4 volumes of 1x Laemmli buffer (50 mmol/L of Tris HCl [pH 6.8], 2% SDS, and 10% glycerol) containing 5 mmol/L of dithiothreitol, 0.5 mmol/L of PMSF, 1 mmol/L of sodium vanadate, and 1.0 μL/mL of a protease inhibitor mixture.23 Protein was determined by the BioRad protein assay. Samples were diluted into sample diluting buffer (20 mmol/L of phosphate buffer [pH 7.4] containing 2 mg/mL of BSA, 0.1% Tween 20, and 0.2% sodium azide) for the MPO ELISA, and MPO levels were as described below.

EDTA plasma samples were obtained from normal weight pre-eclamptic (n=11; 35.5 weeks gestation) and gestationally age-matched at delivery control normal pregnancies (n=13; 36 weeks gestation) with no sign of infection but delivering early. A second set of control subjects (n=17) with 2 blood samples, 1 during gestation (32.1 week) and a second near delivery (38 weeks), was used for comparison of MPO levels in the circulation during pregnancy and preeclampsia. Clinical data for these patients are provided in Table 2. MPO concentrations were measured using ELISA. The accuracy of measuring MPO in previously frozen EDTA plasma was confirmed using MPO ELISA assays from 2 companies (Calbiochem and Assay Designs). Our preliminary experiments indicated that EDTA plasma yielded comparable results to heparinized plasma or spiked plasma using fresh or 1 freeze-thaw plasma. The Calbiochem ELISA standard curve was linear from 1 to 25 ng of MPO per milliliter. The sensitivity of the assay was 1.5 ng/mL with interassay and intra-assay variation of 8%. The Assay Designs (ELISA) standard curve was linear from 0.39 to 12 ng of MPO per milliliter with a sensitivity of 0.13 ng/mL with interassay variation of 7% and intra-assay variation of 5%.

Immunohistochemistry was used to determine MPO localization in the placenta from control normal pregnancies (n=7) and pre-eclamptic pregnancies (n=5) using previously published protocols. Patient data for these samples are provided in Table 3 with the exception of early pregnancy samples from pregnancy terminations at 7.5 and 24.0 weeks (n=2 at each time point). These patients had no complications, including normal blood pressures. Briefly, placental samples were fixed 3% paraformaldehyde for 30 minutes followed by 5%, 10%, and 15% sucrose PBS, then embedded in OCT.

### Table 1. Patient Data for Placental Samples

<table>
<thead>
<tr>
<th>Characteristic (Placental Extracts)</th>
<th>Preeclampsia (n=27)</th>
<th>Normal Pregnant (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>28.4±1.8*</td>
<td>23.1±1.1</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>25.5±1.3*</td>
<td>23.06±1.07</td>
</tr>
<tr>
<td>Weeks gestation at delivery</td>
<td>36.4±0.6</td>
<td>38.8±0.39</td>
</tr>
<tr>
<td>Blood pressure &lt;20 weeks gestation, mm Hg</td>
<td>118±2</td>
<td>112±2</td>
</tr>
<tr>
<td>Systolic</td>
<td>71±2</td>
<td>68±1</td>
</tr>
<tr>
<td>Diastolic</td>
<td>159±7*</td>
<td>118±3</td>
</tr>
<tr>
<td>Predelivery blood pressure, mm Hg</td>
<td>96±3*</td>
<td>72±1</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Data are means±SD. *P<0.05 for preeclampsia vs normal pregnancy.

### Table 2. Patient Data for Plasma Samples

<table>
<thead>
<tr>
<th>Characteristic (Plasma)</th>
<th>Preeclampsia (n=11)</th>
<th>Normal Pregnant (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22±2</td>
<td>27±8</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>25±1</td>
<td>23±8</td>
</tr>
<tr>
<td>Weeks gestation at delivery</td>
<td>36±1*</td>
<td>37±1</td>
</tr>
<tr>
<td>Weeks gestation at blood collection</td>
<td>35±1</td>
<td>36±2</td>
</tr>
<tr>
<td>Blood pressure &lt;20 weeks gestation, mm Hg</td>
<td>118±3</td>
<td>108±4</td>
</tr>
<tr>
<td>Systolic</td>
<td>72±3</td>
<td>77±4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>165±4*</td>
<td>122±6</td>
</tr>
<tr>
<td>Predelivery blood pressure, mm Hg</td>
<td>95±3*</td>
<td>84±5</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Data are means±SD. *P<0.05 for preeclampsia vs normal pregnancy.
Samples were stored at −80°C until sectioned. Anti-MPO antibody (rabbit polyclonal, Calbiochem, La Jolla, CA) at a 1:200 dilution was used to localize MPO.24–26 The primary antibody was omitted as a negative control. Gestational increases in MPO localization in the floating villi from normal pregnancy placentas were used as a positive control.23 Cytokeratin was stained at 1:200 antibody dilution (rat monoclonal IgG).26

For heparin treatment of patients, 16 women (age: 25 ± 1.4 years; 365 ± 38 postpartum) with previous normal pregnancies at a mean of 1 year ± 3.8 days and 15 women with previous preeclamptic pregnancies (age: 29 ± 4 years; 388 ± 30 days postpartum) with normal blood pressures and body mass index were recruited for treatment with heparin (summary patient data in Table 4). All of the women fasted overnight, and initial blood specimens were collected via an 18-gauge intravenous line; this is the “preheparin” sample. If hematocrit fell to <35%, platelets were normal (hematocrit: 35%; platelets: >100 000), women received injection of 75 IU of heparin per kilogram of body weight intravenously. After 15 minutes, 20 mL of venous blood was collected as the “postheparin” sample. Samples were stored at −70°C until use.

Statistical comparisons were by unpaired Student’s t test, 1- or 2-way ANOVA, or Dunn’s test as required, and means±SEs are reported. P values <0.05 were considered significant.

### Results

#### MPO in Placental Extracts

Placental extracts were prepared from preeclamptic (n=27; 36.4 weeks gestation) and control (n=43; 39.1 week gestation) placentas. MPO concentrations in placental extracts from women with preeclampsia were significantly higher than in normal control subjects (546±62 versus 347±32 ng/mL; Figure 1A; P=0.025). Based on previous reports that MPO levels in the placenta increase slowly with gestation, we limited our normal pregnancy group to match for gestational age of the sample (n=16; gestational age 37.5±0.3 weeks) and again found MPO concentrations in placental extracts from normal pregnancy to be much lower (286.5±66 ng/mL; P=0.009).

#### Placental Immunolocalization of MPO

The localization of MPO within the placentas from normal and preeclamptic pregnancies was determined by immunohistochemical analysis. High levels of MPO were found in basal plates from 4 of 5 preeclampsia placentas compared with normal pregnancy placentas, where high levels were found in 7 of placentas (representative slides are shown in Figure 1B, panel D versus B). In the basal plate of women, degenerated cytotrophoblasts were MPO positive; however, in the preeclampsia basal plate, there was also strong staining of cytotrophoblasts and some as-yet-unidentified maternal cells. In the floating villi, MPO levels increased through gestation in normal pregnancies, and this increase was then compared with the MPO in placentas from patients with preeclampsia. Two third-trimester preeclampsia placentas had MPO staining greater than normal third-trimester placentas, and the remaining 3 preeclampsia placentas had MPO staining greater than normal term (Figure 1C, panel D versus B). In the floating villi, MPO staining increased through gestation in the placentas from normal pregnancies, consistent with previous work.27 This is shown in a representative placental sample at 7.5 weeks gestation (Figure 1C, panel B) in comparison with 37.0 weeks (Figure 1C, panel D). Figure 1C (panel F) demonstrates the increased MPO staining intensity in floating villi from a preeclampsia placenta delivered at 26 weeks gestation. Cell types in the villi staining positive for MPO were infiltrating white blood cells (cytokeratin-negative, CD45-positive, and MPO positive cells, shown in the inset of Figure 1C, panel D) and syncytiotrophoblasts (cytokeratin and MPO positive cells). The positive staining of syncytiotrophoblasts was much greater in villi from preeclampsia placentas.

#### MPO in Pregnancy Plasma

EDTA plasma samples from gestationally age-matched at delivery preeclamptic (n=11; 35.5 weeks gestation) and control normal pregnancies (n=13; 36.1 weeks gestation) were obtained for the determination of plasma MPO. Clinical data are provided in Table 2. Plasma MPO levels were 3-fold higher in patients with preeclampsia compared with gestationally age- at delivery-matched normal control subjects (36.6±7.6 versus 11.0±3.1 ng/mL; P=0.003; Figure 2A) by ELISA (Calbiochem). Controlling for gestational age or protein levels did not influence these results. A second set of control patients with 2 samples collected during gestation (20 to 36 weeks) and at term were identified and used to determine whether MPO levels in plasma changed with normal gestation (Figure 2B). These samples were again compared with samples from patients with preeclampsia using a second ELISA assay system (Assay Designs). Plasma MPO levels were not significantly different earlier in gesta-
tion compared with term in normal pregnancies (2.0±0.8 versus 5.0±1.4 ng/mL), whereas MPO in preeclampsia plasma was significantly elevated (32.5±11 ng/mL; Figure 2B). We did not find any trend of increasing plasma MPO levels during gestation to term in the normal pregnancies. In normal pregnancy samples, 36% of patients had no change in MPO with resampling, 27% of patients had increased MPO levels, and 37% had decreased MPO levels with the second measurement. MPO concentrations measured by ELISA for preeclampsia patients were not significantly different between assays.

**MPO in Postpartum Plasma**

To determine the potential of MPO to bind to the vasculature of healthy reproductive-age women and whether preeclampsia-associated elevations in circulating MPO could be detected postpartum, we examined MPO levels in plasma from women postpregnancy before and after a heparin infusion (clinical data provided in Table 4), which would release heparin sulfate–bound MPO into the circulation. Heparin infusion resulted in a 5-fold increase in plasma MPO levels in the circulation 18.5±4 to 103.7±8 (n=16; P<0.001; Figure 3A) in women with normal pregnancies and 20.1±5 to 114±10.3 in women with previous preeclampsia (n=15; P<0.001; Figure 3B). There was no significant difference in plasma MPO levels or in the heparin-releasable levels in women with previous preeclamptic pregnancies compared with women with previous normal pregnancies at ~1 year postpartum.

**Figure 1.** A, MPO levels in placental extracts. MPO levels were determined in placental extracts from patients with preeclampsia (n=27) and normal pregnancies (n=43). MPO levels were significantly greater in placental extracts from preeclampsia (546±62 ng/mL) vs normal control subjects with (287±66 ng/mL) or without (347±32 ng/mL) controlling for gestational age. Expressing MPO levels as nanograms per milliliter of extract or nanograms per milligram of protein did not change these results. MPO was examined immunohistochemically in basal plate (B) and floating villi (C) from placental samples. In B, the basal plates from normal pregnancy placenta at 37 weeks and from preeclampsia placenta (26 weeks) are shown with cytokeratin staining in A and C. MPO immunolocalization in normal pregnancy is shown in B and in preeclampsia (D). There was much greater MPO staining in the basal plate of preeclampsia vs normal pregnancy placenta. In the floating villi (C), A, C, and E are cytokeratin staining of samples from a 7.5-week normal pregnancy (A), 37.0-week normal pregnancy (C), and preeclampsia placentas (E). In floating villi from normal pregnancies, MPO staining increased with gestation from 7.5 weeks (B) to 37.0 weeks (D), whereas the preeclampsia placental sample had the highest levels of staining (F). MPO-positive cells were identified as primarily infiltrating white blood cells (cytokeratin-negative cells, shown in the inset of D) in normal pregnancy and white blood cells and syncytiotrophoblast (cytokeratin and MPO positive cells) in preeclampsia. Magnification is ×100 with a scale of size as indicated on the figures.
MPO levels were significantly increased in placental extracts from women with preeclampsia. Placental MPO levels have been shown to increase with gestational age in the placenta for normal pregnancies.\(^3\) MPO levels were significantly elevated when compared with gestationally age-matched placental samples without preeclampsia or normal term placental samples. The normal pregnancy data were limited to a sample set with matching gestational age to the preeclampsia samples. The normal pregnancy data indicate that there is no long-term mechanism of inflammation present in patients with preeclampsia that causes higher MPO levels compared with women who had a normal pregnancy. These postpartum data also showed that, by 1 year postpartum, there was no significant difference in initial or heparin-releasable MPO in patients with preeclampsia that causes higher MPO levels compared with women with previous normal pregnancies, which remains in the postpartum period.

The previous data on circulating MPO levels in patients with preeclampsia were conflicting;\(^2\) our data agree with studies reporting increased MPO in the circulation of women with preeclampsia.\(^1,3\) Unlike the placenta, circulating MPO levels did not significantly increase with gestation. The most plausible source of MPO in the circulation is activated inflammatory cells in either the circulation or tissue. We speculate that MPO may contribute to the oxidative damage reported in the endothelium of women with preeclampsia.

Once in the circulation, MPO can become sequestered in the subendothelial space through a process of heparin glycosaminoglycan-dependent binding and endothelial transcytosis, resulting in the accumulation of MPO in the endothelial cell matrix.\(^12\) There it can be a potent source of reactive oxygen and nitrogen species. Functional significance of the vascular-associated MPO has been shown in patients undergoing coronary angiography for treatment of coronary artery disease. Baldus et al\(^3\) found no significant difference in baseline plasma MPO levels, but on heparin infusion to release MPO from heparin glycosaminoglycan binding sites, circulating MPO levels were significantly higher in patients with coronary artery disease. Heparin infusion was also associated with improved NO-mediated endothelial function using flow-mediated dilation and responsiveness to acetylcholine. The heparin infusion was associated with a decrease in MPO burden in the vasculature of these patients.\(^3\) We sought to determine the levels of heparin released in young women to assess the potential relevance of this mechanism in healthy patients and also to validate the determination of MPO in EDTA plasma samples from healthy patients. Our data also showed that, by 1 year postpartum, there was no significant difference in initial or heparin-releasable MPO in the circulation of previously preeclamptic women compared with women who had a normal pregnancy. These postpartum data indicate that there is no long-term mechanism of inflammation present in patients with preeclampsia that causes higher MPO levels compared with women with previous normal pregnancies, which remains in the postpartum period.

**Perspectives**

Elevations in MPO have not only been associated with cardiovascular events but have also been shown to have predictive power in high-risk patients.\(^9\) Women with preeclampsia have numerous symptoms (hypertension, inflammation, and endothelial dysfunction) during pregnancy that can be associated with cardiovascular risk factors, and elevations in MPO in these patients may not only index the cardiovascular involvement of this disorder but may also play...
We thank Dr Susan J. Fisher for her assistance with the immuno- histochemical localization of MPO in the placenta.

Sources of Funding
This study was supported by grants HD-30367, MO1RR00056, and RO1 HL64144.

Acknowledgment
We thank Dr Susan J. Fisher for her assistance with the immuno- histochimical localization of MPO in the placenta.

Disclosures
None.

References


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Hypertension. 2008;52:387-393; originally published online June 30, 2008; doi: 10.1161/HYPERTENSIONAHA.107.107532

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/52/2/387

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