Myeloperoxidase in Hypertensive Disorders of Pregnancy and Its Relation With Nitric Oxide

Lilliam Rocha-Penha, Mayara Caldeira-Dias, José Eduardo Tanus-Santos, Ricardo de Carvalho Cavalli, Valéria Cristina Sandrim

Abstract—Elevated levels of myeloperoxidase have been demonstrated in women with preeclampsia where it may contribute to endothelial dysfunction mediated, in part, by nitric oxide impairment. In this study, we investigated myeloperoxidase in hypertensive disorders of pregnancy and its contribution to the impairment of the vasodilator nitric oxide. We found higher levels of myeloperoxidase in supernatant from human umbilical vein endothelial cells cultures incubated with plasma from preeclampsia group compared with healthy pregnant women. Further, we measured plasma concentration and activity of myeloperoxidase in 219 healthy pregnant women, 130 gestational hypertension (on antihypertensive therapy or not), and 143 preeclampsia patients (on antihypertensive therapy or not). We found that patients with preeclampsia and gestational hypertension without antihypertensive treatment showed higher levels and activity of this enzyme, respectively. Moreover, the inhibition of myeloperoxidase activity in vitro improved nitric oxide bioavailability. Our results indicate a higher cardiovascular risk in pregnant women with hypertensive disorders, and that active myeloperoxidase may play a role in endothelial dysfunction in these conditions by impairment of nitric oxide availability. Besides, the use of antihypertensive drugs seems to decrease enzyme levels suggesting a new protective feature for these drugs. (Hypertension. 2017;69:1173-1180. DOI: 10.1161/HYPERTENSIONAHA.116.08854.) ● Online Data Supplement

Key Words: cardiovascular diseases ■ heparin ■ human umbilical vein endothelial cells ■ nitric oxide ■ pregnancy

Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, affect about 10% of pregnancies and are the major cause of maternal and prenatal morbidity and mortality.1,2 Although the cause of preeclampsia remains unknown,3 it is thought that an initial stage of poor placentation with posterior ischemia stimulates the release of soluble factors by the placenta into maternal circulation, which lead to a second stage of the disorder characterized by maternal endothelial dysfunction.4–6 The incubation of plasma or serum from pregnant women with preeclampsia on endothelial cells has been used to understand the link between these stages.2–11

Myeloperoxidase is a lysosomal enzyme mainly produced and released by activated neutrophils and, in lesser extent by monocytes, which produces highly oxidizing substances from hydrogen peroxide and halogens.12,13 This enzyme is a potent trigger to vascular injury and has been related to pathogenesis and progression of several cardiovascular diseases, mainly because of its proinflammatory and oxidative properties.14,15 Notably, myeloperoxidase is associated with impairment of nitric oxide (NO) bioavailability,15–17 a molecule produced by endothelial cells with functions such as vasodilation and anticoagulation, which is known to be reduced in preeclampsia.18,19 Although already reported20,21 the possible contribution of myeloperoxidase to endothelial dysfunction, no previous study examined the effect of endothelial cell incubation with plasma from pregnant with hypertensive disorders on myeloperoxidase levels or activity.

The findings on plasma/serum levels of myeloperoxidase in preeclampsia are controversial22–28 reporting higher or similar levels among groups. Besides, these studies enrolled a relatively small number of patients (ranging from 11 to 61 subjects), none included patients with gestational hypertension, used different types of blood collection tubes, did not described the antihypertensive drugs in use at sampling, and the results are only related to myeloperoxidase activity or protein levels.22–28

Considering myeloperoxidase characteristics and abilities and the hallmarks of hypertensive disorders of pregnancy, we hypothesized that myeloperoxidase would be elevated in subjects with hypertensive disorders of pregnancy and would play an important role in endothelial dysfunction through its influence on NO. Therefore, in this study, we (1) evaluated plasma myeloperoxidase concentration and activity in healthy, hypertensive, and preeclamptic pregnant women and investigated preanalytical influences comparing these parameters between plasma from heparinized and EDTA tubes, to
facilitate comparison within other works and (2) examined whether inhibition of myeloperoxidase may modulate NO availability in vitro.

Materials and Methods
More details for Materials and Methods in the online-only Data Supplement.

Study Workflow
The schematic diagram of the study workflow is shown in Figure S1 in the online-only Data Supplement. We firstly performed a multiplex assay to investigate the concentration of 8 proteins related to cardiovascular risk in supernatant of endothelial cells after 24-hour incubation with plasma from healthy pregnant women, gestational hypertensive, and preeclamptic women. Myeloperoxidase was the only differently expressed biomarker between healthy pregnant women and preeclampsia. Therefore, we proceeded with the following experiments with this biomarker: we measured both myeloperoxidase concentration and activity in plasma from the 3 groups and, as some studies indicated differences in myeloperoxidase levels between blood collection tubes,29,35 we also compared these parameters between plasma samples collected simultaneously in heparinized and EDTA tubes. Once myeloperoxidase is able to consume the vasodilator NO, we examined whether inhibition of its enzymatic activity would improve NO availability in vitro.

Patients
The subjects were recruited from the clinics of the Department of Obstetrics and Gynecology of the Hospital das Clínicas de Ribeirão Preto, University of São Paulo. All participants provided written informed consent, and the study was approved by the Institutional Review Boards of the Hospital das Clínicas de Ribeirão Preto, University of São Paulo (reference 4682/2006, approved date June 20, 2006). Hypertensive disorders of pregnancy were defined following guidelines of National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy.33 Patients with hemostatic abnormalities, chronic hypertension, diabetes mellitus, fetal abnormalities, cancer, twin pregnancy, cardiovascular, autoimmune, renal, and hepatic diseases were excluded from this study. At the time of clinical attendance, maternal venous blood samples were collected in tubes containing EDTA and heparin as anticoagulants, simultaneously. The tubes were rapidly centrifuged (1000 g for 3 min) and plasmas were stored at −70°C until analysis.

Cell Culture and Plasma Incubation
Human umbilical vein endothelial cell line (CRL 2873) was cultured until reaching 80% confluence and then incubated in medium with 20% (v/v) of heparinized plasma from healthy pregnant women, gestational hypertensive, or preeclamptic patients for 24 hours. Supernatant of these cultures was used to perform the multiplex assay.

To investigate the relation between myeloperoxidase and NO availability in vitro, plasma samples were incubated with increasing concentrations (5, 20, and 50 µmol/L) of myeloperoxidase inhibitor 4-aminobenzoic acid hydrazide, prior addition in the culture medium, and 24-hour incubation.

Human Cardiovascular Disease Panel
Protein levels from culture supernatant were assessed in simplicate by Luminex xMAP technology using human cardiovascular disease panel 2 (catalogue HCVD2MAG-67K; Millipore, Billerica, MA) composed by the following analytes: myeloperoxidase, growth differentiation factor 15, myoglobin, P-selectin, soluble intercellular adhesion molecule-1, neutrophil gelatinase–associated lipocalin, ADAMTS-13 (A Disintegrin and Metalloproteinase With Thrombospondin Type 1 Repeats 13), and serum amyloid A, according to the manufacturer’s instructions. Luminex panel were analyzed on a MAGPIX multiplex reader (Luminex, Austin, TX). Intra-assay was 2.4%.

Myeloperoxidase Concentration and Activity
Myeloperoxidase concentration was evaluated in duplicate in plasma samples (from EDTA and heparin tubes) using a commercial enzyme-linked immunosorbent assay kit. Standard range was between 62.5 and 4000.0 pg/mL and, inter- and intra-assay were 7.5% and 5.1%, respectively.

Myeloperoxidase activity was determined in duplicate by measuring tetramethylbenzidine oxidation, based on the method described by Bradley et al.36 To determine whether the obtained activity was derived from myeloperoxidase, samples were incubated with 500 µmol/L of a specific and irreversible myeloperoxidase inhibitor (4-aminobenzoic acid hydrazide) at 37°C for 30 minutes prior addition of substrate system. Inter- and intra-assay were 1.3% and 1.1%, respectively.

Nitrite Quantification
Nitrite quantification from culture supernatant was performed in duplicate using Griess reagents. Samples (50 µL) were incubated with 50 µL of 1% sulfuramidine solution in 5% phosphoric acid for 10 minutes protected from light. Then, 50 µL of 0.1% N-(1-Naphthyl)-ethylenediamine dihydrochloride solution were added followed by 10-minute incubation. Optical density was determined in a microplate reader at 540 nm. A standard curve were generated by incubation of nitrite solutions (0.46–29.5 µmol/L mL−1) with the previous reagents. Intra-assay was 9.1%.

Plasma nitrite were quantified in triplicate by an ozone-based chemiluminescence assay as previously described.19

Statistical Analysis
Data distribution was analyzed using D’Agostino-Pearson omnibus normality test. Categorical variables were compared by χ² tests. Continuous variables were compared by Student t test, ANOVA followed by Tukey test (for normally distributed variables), or Mann–Whitney and Kruskal–Wallis followed by Dunn multiple comparison test (for not normally distributes variables). Correlations were assessed by Pearson or Spearman correlation, as appropriated. The analyses were performed with GraphPad Prism for Windows, version 6.01 (GraphPad Software, San Diego, CA). For all tests, a probability value of P ≤ 0.05 was considered significant, except for correlation where was considered P ≤ 0.01.

Results
In Vitro Quantification of Proteins Related to Cardiovascular Risk
At first, we quantified the concentration of 8 biomarkers related to cardiovascular diseases (myeloperoxidase, growth differentiation factor 15, Myoglobin, P-selectin, soluble intercellular adhesion molecule-1, neutrophil gelatinase–associated lipocalin, ADAMTS-13, and serum amyloid A) in culture supernatant of endothelial cells after 24-hour incubation with plasma from healthy pregnant women, gestational hypertensive, and preeclamptic women (Figure S2). General characteristics of pregnant women enrolled in this study are shown in Table S1. As expected, systolic and diastolic blood pressure were higher in hypertensive disorders of pregnancy when compared with healthy pregnant women (P<0.0001), whereas gestational age at delivery and newborn weight were lower in preeclampsia group (P=0.0007 and P=0.009, respectively).

Patients with preeclampsia also presented higher proteinuria (P<0.0001) and, as some studies indicated differences in myeloperoxidase levels among these groups (5.20, and 50 µmol/L), we proceeded with the following experiments with this biomarker: we measured myeloperoxidase concentration and activity in plasma from the 3 groups and, as some studies indicated differences in myeloperoxidase levels between blood collection tubes,29,35 we also compared these parameters between plasma samples collected simultaneously in heparinized and EDTA tubes. Once myeloperoxidase is able to consume the vasodilator NO, we examined whether inhibition of its enzymatic activity would improve NO availability in vitro.

Myeloperoxidase Concentration and Activity
Myeloperoxidase concentration was evaluated in duplicate in plasma samples (from EDTA and heparin tubes) using a commercial enzyme-linked immunosorbent assay kit. Standard range was between 62.5 and 4000.0 pg/mL and, inter- and intra-assay were 7.5% and 5.1%, respectively.

Myeloperoxidase activity was determined in duplicate by measur-
Concentration and Activity of Myeloperoxidase in Plasma Samples

On the basis of the in vitro results, we next explored circulating levels of myeloperoxidase in the 3 groups of pregnant women. General characteristics of women enrolled in this part of the study are shown in Table. As expected, systolic and diastolic blood pressure were higher in hypertensive disorders of pregnancy when compared with healthy pregnant women ($P<0.0001$). As indicated by mean values in Table, most subjects had mild hypertension, although a few patients had blood pressure levels $>140/90$ mmHg (data not shown). However, subjects with hypertensive disorders had elevated body mass index ($P<0.0001$) and lower gestational age at delivery ($P<0.0001$) than healthy pregnant women group and preeclampsia group lower than gestational hypertension. Also higher levels of sFlt-1 (soluble fms-like tyrosine kinase-1; $P<0.0001$) and lower newborn weight ($P<0.0001$) were observed in preeclampsia compared with healthy pregnant women.

Plasma myeloperoxidase concentration and activity in healthy pregnant women, gestational hypertensive, and preeclampsia are shown in Figure 1, both subgrouped in women on or without antihypertensive treatment at time of blood collection. Myeloperoxidase levels in gestational hypertension (17.4±2.1 ng/mL) and preeclampsia (15.4±1.4 ng/mL) on treatment were lower than healthy pregnant women group (27.5±2.2 ng/mL; Figure 1A). Interestingly, preeclampsia without antihypertensive treatment (51.2±3.8 ng/mL) presented higher levels of myeloperoxidase compared with preeclampsia and gestational hypertension on antihypertensive treatment and compared with healthy pregnant women and gestational hypertension (19.4±2.8 ng/mL) without treatment (Figure 1A). About myeloperoxidase activity, no differences were observed between the 3 groups on antihypertensive treatment (Figure 1B; $P=0.8$). However, among healthy pregnant women and the groups without treatment, gestational hypertension (192.1±21.2 U/L) showed higher enzymatic activity when compared with healthy pregnant women (146.1±5.7 U/L) and preeclampsia (124.7±6.7 U/L; Figure 1B). Worth noting that, despite the small number of subjects (2.2% in healthy pregnant women, 1.8% in gestational hypertensive, and 11.1% in preeclampsia group) with intrauterine growth restriction, no differences were observed in the myeloperoxidase levels nor activity after exclusion of these subjects (Figure S3).

Considering that some substances may react with the substrate (tetramethylbenzidine) of myeloperoxidase activity assay, we examined plasma myeloperoxidase activity in all groups in the presence of 4-aminobenzoic acid hydrazide, a specific and irreversible inhibitor of myeloperoxidase. This assay may provide us a confirmation that the values obtained in our enzymatic assays were derived only from myeloperoxidase activity. The incubation of plasma from healthy pregnant women, gestational hypertensive, and preeclampsia women with 4-aminobenzoic acid hydrazide significantly reduced enzymatic activity in all groups ($P<0.0001$) in $>90\%$, indicating that mostly of the activity previously assessed in these groups were derived from myeloperoxidase (Figure S4).

About the correlations with clinical parameters (Table S4), we found a positive correlation between enzyme levels and red blood cells (Figure S5A; $P=0.003$; $r=0.4$) and hemoglobin (Figure S5B; $P=0.01$; $r=0.2$) in healthy pregnant women group.

Influence of Anticoagulant Present in Blood Collection Tubes on Myeloperoxidase Analysis

Although not yet evaluated in preeclampsia studies, previous findings showed differences in myeloperoxidase concentration between blood collected in tubes containing heparin and ethylenediaminetetraacetic acid (EDTA) as anticoagulants. Therefore, we have also measured enzyme levels and activity in plasma from blood collected in tubes with heparin from healthy pregnant women, gestational hypertension, and preeclampsia groups and compared with results from EDTA-plasma. Both sample tubes were collected simultaneously. We observed lower levels of the enzyme in plasma from heparin tubes compared with plasma from EDTA tubes in all groups (Figure S6A; $P<0.04$). Comparing enzymatic activity, plasma from heparin tubes showed important reduction of activity levels than plasma from EDTA tubes in all groups (Figure S6B; $P<0.0001$). Because of the enzymatic activity reduction in samples from heparin tubes, we then investigate whether the heparin from blood collection tubes had the ability to inhibit myeloperoxidase activity. Figure S7 shows enzymatic activity before and after plasma from EDTA tubes incubation with heparin solution (14 U/mL). Samples collected in EDTA tubes from all groups incubated with heparin solution showed a reduction in enzymatic activity when compared with those without heparin addition ($P<0.03$), suggesting that heparin from blood collection tube may inhibit myeloperoxidase activity in plasma samples.

In Vitro and Plasma Relation Between Myeloperoxidase and NO Bioavailability

Considering that myeloperoxidase is able to consume the vasodilator NO and thus contributes to endothelial dysfunction, we examined whether the inhibition of myeloperoxidase activity could improve the NO bioavailability in endothelial cells. Figure 2A shows that addition of plasma after myeloperoxidase inhibition in cultures first decreased myeloperoxidase activity in at least 30% at the lower concentration (5 μmol/L) and 41% at higher concentration (50 μmol/L) and significantly increased nitrite concentrations in culture supernatant in all 3 groups studied (Figure 2B; $P<0.01$). Of note, the groups showed an increase trend in nitrite concentrations proportional to myeloperoxidase inhibition. Moreover, although all groups had an increase in nitrite concentrations after myeloperoxidase inhibition, this increase showed to be lower in preeclampsia. Next, we verify whether plasma levels of nitrite were also correlated with plasma concentration and activity of
myeloperoxidase (Table S5). However, we found no significant correlations for all groups ($P>0.05$).

**Discussion**

This study was the first to report higher levels of myeloperoxidase in the supernatant of human umbilical endothelial cells after incubation with plasma from preeclampsia patients. As previously shown, the ischemic placenta releases several bioactive factors in maternal circulation, and these factors lead to oxidative stress, which may stimulate myeloperoxidase expression by the endothelial cells. Higher levels of myeloperoxidase have been related with elevated risk for cardiovascular diseases through its ability to enhance neutrophil generation of reactive species and degranulation and to bind endothelial cell surface and accumulate in subendothelial space contributing to endothelial dysfunction by impairment of NO. Therefore, we examined whether myeloperoxidase activity inhibition in vitro would improve NO availability. We found that inhibition of myeloperoxidase enhances nitrite levels, a marker of NO availability, which suggests that the consumption of NO by myeloperoxidase depends on its catalytic activity. Notably, this finding might open a new therapeutic possibility for the application of substances that modulate myeloperoxidase activity, as some phytochemicals, to improve vasodilator offer and, thus, ameliorate the endothelial dysfunction. Moreover, although reduced circulating nitrite levels in hypertensive disorders of pregnancy, we did not find correlation between plasma nitrite levels and circulating myeloperoxidase concentration/activity. However, this analysis might be complicated by the high complexity of plasma and by some factors that are able to influence in circulating nitrite levels as diet and metabolic transformations as already discussed by our group and others. Diet was shown to contribute more with the nitrate content and our samples were rapidly manipulated to minimize metabolic influences on nitrite levels.

Discordant findings for circulating myeloperoxidase levels in preeclampsia have been reported elsewhere, and to facilitate comparison of the results, these studies are detailed in Table S6. Some authors, as Gandley et al.
al26 and Mellembakken et al.28 reported higher levels of myeloperoxidase in plasma from patients with preeclampsia, and Gandley et al26 also demonstrated similar results in placental samples. In turn, Bowen et al23 and Karacay et al24 observed similar levels in preeclampsia and eclampsia patients and in subjects with mild and severe forms of preeclampsia compared with healthy pregnant women, respectively. Conversely, Hung et al22 did not observe differences in plasma or placental levels of myeloperoxidase, but the anticoagulant used in blood collection was not specified and might influence the comparison of results. The differences among studies may be attributed to several factors such as the inclusion of different forms of preeclampsia or its complications24,28 and the lack of report of antihypertensive drugs use or treatment with heparin.

Similarly to some authors cited above, here we observed higher plasmatic levels of myeloperoxidase in preeclampsia patients without antihypertensive treatment. The release of bioactive factors by the ischemic placenta in preeclampsia contributes to an increase in blood pressure and oxidative stress what may result in several damages on maternal organism. Antihypertensive treatment is indicated in some cases to reduce maternal and fetal outcomes and to prevent neuro and cardiovascular events related to the elevated blood pressure.33,45 Methyldopa is the main drug indicated in these cases, and it is a centrally α2-adrenergic agonist, which decreases the release of norepinephrine. Studies demonstrated the ability of sympathetic system in modulate the immune system,46 and that catecholamines contribute to release of cytokines 47,48 and lysosomal enzymes from immune cells.49 Therefore, the lower myeloperoxidase in preeclampsia patients on antihypertensive treatment observed in our results might be related to a decrease in leucocytes activation because of methyl-dopa action on norepinephrine. Concerning the influence of intrauterine growth restriction, when these subjects were excluded from the analysis, no differences were observed in myeloperoxidase levels/activity. Similarly, Hung et al22 did not found difference in myeloperoxidase levels between groups with and without intrauterine growth restriction, and Stepan et al50 also found no difference in the enzyme levels in patients that subsequently developed preeclampsia or intrauterine growth restriction, suggesting that the neutrophil activation and release of myeloperoxidase might be a secondary effect.

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**Figure 1.** Myeloperoxidase (MPO) concentration and activity in plasma from EDTA tubes from healthy pregnant women (HP, white columns), gestational hypertensive (GH, grey columns) and preeclampsia (PE, black columns) patients subgrouped in patients on (+) and without (−) antihypertensive treatment. A, MPO concentration in treated groups showed to be higher in HP (n=204; P=0.02) than in GH (n=73) and PE (n=80). In groups without treatment, PE (n=31) showed higher levels than GH (n=32) and HP (n=204; P<0.0001). B, MPO activity showed no significant differences in treated groups (P=0.8). Boxplot indicates median [min–max]. Comparison between groups were by Kruskal–Wallis followed by Dunn post test. *P<0.05 vs HP, †P<0.05 vs GH (−), ‡P<0.05 vs PE (−), §P<0.05 vs PE (+).

**Figure 2.** Myeloperoxidase (MPO) activity and nitrite concentration in culture supernatant after 16-h incubation with plasma from heparin tubes from healthy pregnant women (HP, n=5), gestational hypertension (GH, n=5) and preeclampsia (PE, n=5) without (−, white columns) and after addition of MPO inhibitor (4-aminobenzoic acid hydrazide [ABAH]) at 5 µmol/L (5, grey columns) and 50 µmol/L (50, black columns). A, MPO activity after incubation with either plasma (−) or plasma+ABAH. Inhibitor addition decreased activity in all groups in at least 30% and 40.5% at 5 and 50 µmol/L, respectively. B, Nitrite concentration after incubation cited previously. Nitrite levels increased after MPO inhibition in a least of 249% and 266% at 5 and 50 µmol/L, respectively. Boxplot indicates median [min–max]. Comparisons between groups were by Kruskal–Wallis followed by Dunn post test. *P<0.05 vs − from respective group.
Kurdoglu et al. and Noyan et al. evaluated myeloperoxidase activity, but not concentration, and observed higher activity in the mild and severe form of preeclampsia and eclampsia, respectively. Again, none reported any treatments, and they used different sample types (plasma from EDTA tubes and serum, respectively). To our knowledge, we are the first to investigate myeloperoxidase activity in gestational hypertension, and here we show higher myeloperoxidase activity in this group when compared with healthy pregnant women and preeclampsia, despite the influence of antihypertensive therapy. These antihypertensive drugs showed a discrete influence over enzymatic activity in gestational hypertension, and the slight decrease can be associated with the type of antihypertensive drug used, as a few patients of this group were being treated with hydralazine, which has been reported to inhibit myeloperoxidase activity. Unlike the concentration, myeloperoxidase activity on preeclampsia patients was not increased what can be a result of the higher levels of an endogenous inhibitor of myeloperoxidase, ceruloplasmin, as already reported.

When analyzing correlations between myeloperoxidase and clinical parameters, we observed a positive correlation between myeloperoxidase concentration and erythrocytes, as well as hemoglobin. This result can be associated to the ability of red blood cells to act as carriers of this enzyme in circulation because of the cationic characteristic of the enzyme what contribute to its binding to the cell membrane, as demonstrated elsewhere. Although expected based on reports from Shukla et al. that reported increased myeloperoxidase in systemic vasculature of obese and preeclampsia women, no significant correlation was observed between myeloperoxidase and BMI. However, this difference may be related to the type of samples and methods applied between our studies.

Myeloperoxidase has been implicated in a wide range of pathologies, such as cancer, cardiovascular, neurodegenerative, renal and lung diseases, and other chronic inflammatory conditions. However, analytic procedures may influence the precision of myeloperoxidase as a biomarker, and, to date, there is no standardized method for myeloperoxidase evaluation. We found an increase in myeloperoxidase concentrations on plasma from EDTA tubes compared with samples from heparin tubes, which is in line with other findings. Conversely, other studies show higher myeloperoxidase levels in heparin tubes, what may be explained by different preanalytical handling and quantification methods used in the analysis. For example, in 1 study blood samples were kept at room temperature for nearly 1 hour what contributes to the leakage of myeloperoxidase from neutrophils stimulated by the heparin in the tube, resulting in increased levels in plasma. In another study the quantification was performed through a chemiluminescent immunoassay. To our knowledge, to date, there were no reports that investigated these influences on myeloperoxidase activity. Our results showed a significant decrease in enzymatic activity in samples from heparin tubes, and after plasma incubation with heparin solutions, myeloperoxidase activity was significantly reduced, which indicates an inhibitory capacity of the anticoagulant heparin on myeloperoxidase activity. However, further studies are needed to examine other possible interferences in myeloperoxidase analysis to standardize preanalytical handling and analysis method ensuring greater reliability of the results.

In conclusion, we found higher myeloperoxidase levels in preeclampsia group without antihypertensive treatment, as well as higher activity in gestational hypertension without treatment, thus suggesting a new protective feature for these drugs by decreasing the enzyme release and the damage cause to the endothelium. Besides, inhibition of the enzymatic activity in vitro resulted in an improvement of nitrite availability. These findings suggest a higher risk for cardiovascular diseases in these groups, and that myeloperoxidase may play a role on the endothelial dysfunction, characteristic of these conditions.

**Perspectives**

Here, we showed that antihypertensive therapy might affect the analysis of myeloperoxidase, indicating an interaction between myeloperoxidase and antihypertensive treatment that might suggest a possible protector effect of these drugs on the endothelial damage cause by this enzyme. A deeper investigation is required to identify which antihypertensive drugs has the ability to interact with this enzyme, through which mechanism this happens, and to evaluate a possible protective characteristic against endothelium myeloperoxidase-derived damage. Moreover, the improvement of nitrite concentrations after myeloperoxidase inhibition showed here provide new possibilities for the application of substances that might modulate myeloperoxidase, as some phytochemicals or even antihypertensive drugs, as suggested by our results, to attempt to ameliorate endothelial function. Last, the reduction of myeloperoxidase activity in plasma collected in heparin tubes compared with collect in EDTA tubes strengthens the need of preanalytical handling standards to ensure reliability of the results.

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**Disclosures**

None.

**References**


What Is New?

* Quantification of myeloperoxidase in vitro after incubation with plasma; investigation of myeloperoxidase in gestational hypertensive, influence of antihypertensive therapy on myeloperoxidase, and influence of blood collection tubes in myeloperoxidase activity (EDTA [ethylenediaminetetraacetic acid] versus heparin).

What Is Relevant?

* Hypertensive disorders of pregnancy are related to systemic inflammation, oxidative stress, endothelial dysfunction, and higher cardiovascular risk.
* Myeloperoxidase is able to impair nitric oxide leading to endothelial dysfunction and contributing to hypertension, being widely associated with cardiovascular diseases.

Novelty and Significance

Higher myeloperoxidase levels in preeclampsia and activity in gestational hypertension and inhibition of the enzymatic activity in vitro improved nitrite availability, presence of antihypertensive drugs related to lower myeloperoxidase. All together, our results suggest higher risk for cardiovascular diseases in pregnant hypertensive groups, a role of myeloperoxidase on the endothelial dysfunction by consuming NO and a possible new protector feature of antihypertensive drugs.
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MYELOPEROXIDASE IN HYPERTENSIVE DISORDERS OF PREGNANCY AND ITS RELATION WITH NITRIC OXIDE

Authors’ names and affiliations:

Lilliam Rocha-Penha ¹, Mayara Caldeira-Dias ¹, José Eduardo Tanus-Santos ², Ricardo de Carvalho Cavalli ³, Valéria Cristina Sandrim 1*

1-Department of Pharmacology, Institute of Biosciences of Botucatu, Universidade Estadual Paulista (UNESP), Botucatu, Distrito Rubiao Junior, 18680-000, São Paulo, Brazil

2- Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirao Preto, SP, Brazil.

3- Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirao Preto, SP, Brazil.
DETAILED MATERIAL AND METHODS

Patients
Classification of hypertensive disorders of pregnancy:
Gestational hypertension was defined as increased blood pressure (≥140 mmHg systolic and/or ≥90 mmHg diastolic) on at least two measurements with six hours apart, without proteinuria (<0.3g in 24h) after 20 weeks of gestation. Preeclampsia was defined as increased blood pressure (≥140 mmHg systolic and/or ≥90 mmHg diastolic) on at least two measurements with six hours apart, with significant proteinuria (≥0.3g/24h) after 20 weeks of gestation.

Exclusion criteria:
Hemostatic abnormalities comprise anemia, thrombocytopenia, coagulopathies, microangiopathic hemolysis and disseminated intravascular coagulation. Chronic hypertension comprises patients with diagnosis of hypertension before pregnancy or before 20 weeks gestation. Diabetes was defined following guidelines of Hyperglycaemia and Adverse Pregnancy Outcomes [HAPO] study1.

Cell culture and plasma incubation
Human umbilical vein endothelial cell (HUVEC) line (CRL 2873) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). HUVEC were cultured in DMEM medium (Gibco, CA, USA) supplemented with 10% (v/v) fetal calf serum, 50μg/ml penicillin, 50μg/ml streptomycin and 0,5μg/ml amphotericin B (Gibco) at 37°C in 5% CO2 incubator. Cells were expanded in 75cm² tissue culture flasks (Corning, Costar, Netherlands) and after reaching 80% confluence, they were detached from the flask surface by trypsin/EDTA (0.5/0.2 mg/ml in phosphate-buffered saline, PBS) and split at a 1:3 ratio into 25cm² flasks. At passage 3, HUVEC were re-suspended in DMEM medium and re-plated in 48-well tissue culture plates (Corning, Costar, Netherlands). After reaching 80% confluence, the medium was removed and cells were washed twice in PBS and then incubated in medium with 20% (v/v) of heparinized plasma from healthy pregnant women, gestational hypertensive or preeclamptic patients for 24 hours. Supernatant of these cultures was used to perform the multiplex assay.

To investigate the relation between MPO and NO availability in vitro, plasma samples were incubated with increasing concentrations (5, 20 and 50µM) of MPO inhibitor 4-aminobenzoic acid hydrazide (ABAH; Sigma, St. Louis, MO, USA), prior addition in the culture medium and 24-hours incubation.

Myeloperoxidase concentration and activity
MPO concentration was evaluated in duplicate in plasma samples (from EDTA and heparin tubes) using a commercial enzyme-linked immunosorbent assay (Human Myeloperoxidase DuoSet ELISA - R&D Systems, Minneapolis, MN, USA). Plasma samples were diluted 1:100 in reagent diluent (1% BSA in PBS) and optical density was determined at 450nm using a microplate reader (Synergy 4, BioTek – Winooski, VT, USA). Standard range was between 62.5 – 4,000.0 pg/mL and, inter and intra-assay was 7.5% and 5.1%, respectively.

MPO activity was determined in duplicate by measuring tetramethylbenzidine (TMB) oxidation, based on the method described by Bradley et al.2. For that, 30µL of plasma (1:100) were incubated with 20µL of phosphate buffer and 100µL of liquid substrate system, composed by TMB and hydrogen peroxide (Sigma, St. Louis, MO, USA), at 37°C for 10 minutes, protected from light. After incubation, 100µL of stop solution (sulfuric acid 2N) were added and optical density were determined at 450nm using a microplate reader. A standard curve was generated by incubation of horseradish peroxidase with the previous reagents. The standard range were between 0.153 – 2500 mU/50µL. In order to determine whether the obtained activity was derived from MPO, samples were incubated with 500µM of a specific and irreversible MPO inhibitor (ABAH) at 37°C for 30 minutes prior addition of substrate system. Inter and intra-assay were 1.3% and 1.1%, respectively.

Nitrite quantification
Nitrite quantification from culture supernatant was performed in duplicate using Griess reagents (Sigma, St. Louis, MO, USA): sulfanilamide (cat. S9251), phosphoric acid (cat. V001529), N-(1-Naphthyl)-ethylenediamine dihydrochloride (cat. 33461), sodium nitrate (cat. S2252).

Plasma nitrite were quantified in triplicate by an ozone-based chemiluminescence assay as previously described3 using chemiluminescence NO analyzer. Briefly, 200µL of plasma were added to 8mL of acidified triiodide solution (2.0g of potassium iodide + 1.3g of iodine in 40mL of water with 140mL of acetic acid) and purged with nitrogen in-line with a gas-phase chemiluminescence NO analyzer (Sievers model 280 NO Analyzer – Boulder, CO, USA).
REFERENCES


Table S1. Patients characteristics for in vitro study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy pregnancy (n = 25)</th>
<th>Gestational hypertension (n = 10)</th>
<th>Preeclampsia (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.3 ± 0.9</td>
<td>28.6 ± 1.6</td>
<td>26.2 ± 1.3</td>
</tr>
<tr>
<td>Race (white, %)</td>
<td>68</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td>Prepregnancy BMI (Kg/m²)</td>
<td>28.3 ± 1.2</td>
<td>29.8 ± 1.7</td>
<td>27.8 ± 1.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115.0 ± 2.0</td>
<td>133.2 ± 5.5*</td>
<td>135.2 ± 3.2*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71.8 ± 1.5</td>
<td>87.1 ± 4.8*</td>
<td>86.6 ± 2.6*</td>
</tr>
</tbody>
</table>

Antihypertensive drugs at blood collection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy pregnancy</th>
<th>Gestational hypertension</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Methyldopa (%)</td>
<td>NA</td>
<td>90.0</td>
<td>70.8</td>
</tr>
<tr>
<td>Nifedipine (%)</td>
<td>NA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GA at blood collection (weeks)</td>
<td>37.0 ± 0.4</td>
<td>35.6 ± 1.7</td>
<td>35.2 ± 0.8</td>
</tr>
<tr>
<td>RBC count (x10⁶/mL)</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.3 ± 0.4</td>
<td>11.7 ± 0.3</td>
<td>11.9 ± 0.2</td>
</tr>
<tr>
<td>WBC count (x10³/mL)</td>
<td>10.9 ± 0.9</td>
<td>9.2 ± 0.8</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>76.1 ± 2.3</td>
<td>69.0 ± 2.5</td>
<td>66.9 ± 4.0</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>74.0 (68.0 – 78.0)</td>
<td>79.0 (72.0 – 89.0)</td>
<td>77.5 (67.7 – 82.5)</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>ND</td>
<td>177.7 ± 42.9</td>
<td>654.9 ± 129.6†</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>39.9 ± 0.3</td>
<td>38.5 ± 1.3</td>
<td>36.2 ± 0.8*</td>
</tr>
<tr>
<td>Newborn weight (g)</td>
<td>3385.0 ± 77.0</td>
<td>2832.0 ± 330.7</td>
<td>2645.0 ± 202.3*</td>
</tr>
</tbody>
</table>

Data as mean ± SEM, median (25th – 75th centiles) or percentage. Comparisons between groups were by ANOVA 1-way followed by Tukey’s post test or Mann-Whitney followed by Dunn’s for continuous variables or chi-square test for categorical variables. *P<0.05 vs healthy pregnancy; †P<0.05 vs gestational hypertension. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GA, gestational age; RBC, red blood cells; WBC, white blood cells; NA, not applicable; ND, not determined.
### Table S2. Quality control for human cardiovascular disease panel analytes

<table>
<thead>
<tr>
<th>Quality Control (ng/mL)</th>
<th>MPO</th>
<th>GDF-15</th>
<th>Myoglobin</th>
<th>P-selectin</th>
<th>sICAM-1</th>
<th>NGAL</th>
<th>ADAMTS-13</th>
<th>sAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC1(observed)</td>
<td>0.25</td>
<td>0.01</td>
<td>0.31</td>
<td>3.22</td>
<td>1.21</td>
<td>0.15</td>
<td>16.2</td>
<td>2.74</td>
</tr>
<tr>
<td>QC2 (observed)</td>
<td>3.06</td>
<td>0.08</td>
<td>2.96</td>
<td>31.27</td>
<td>10.4</td>
<td>1.47</td>
<td>150.7</td>
<td>23.9</td>
</tr>
<tr>
<td>QC1 (expected range)</td>
<td>0.14 – 0.7</td>
<td>0.008 – 0.017</td>
<td>0.31 – 0.6</td>
<td>3.2 – 6.6</td>
<td>1.2 – 2.4</td>
<td>0.15 – 0.32</td>
<td>16 – 32</td>
<td>2.7 – 5.6</td>
</tr>
<tr>
<td>QC2 (expected range)</td>
<td>2.4 – 5.1</td>
<td>0.07 – 0.14</td>
<td>2.5 – 5.2</td>
<td>27 – 56</td>
<td>10 – 21</td>
<td>1.3 – 2.7</td>
<td>125 – 259</td>
<td>23 – 48</td>
</tr>
</tbody>
</table>

Data as mean or min. – max. QC represents quality control; MPO, myeloperoxidase; GDF-15, growth differentiation factor 15; sICAM-1, soluble intercellular adhesion molecule-1; NGAL, neutrophil gelatinase associated lipocalin; ADAMTS-13, A desintegrin and metalloprotease with thrombospondin type 1 repeats 13; sAA, serum amyloid A.
Table S3. Ranges of biomarkers concentration for cardiovascular disease panel, referent to Figure 1.

<table>
<thead>
<tr>
<th>Analyte (ng/mL)</th>
<th>Healthy pregnant</th>
<th>Gestational hypertension</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>3.1 (1.9 – 7.2)</td>
<td>4.1 (2.0 – 9.4)</td>
<td>7.9 (3.9 – 14.7)</td>
</tr>
<tr>
<td>GDF-15</td>
<td>3.3 (1.3 – 4.1)</td>
<td>3.7 (1.4 – 4.4)</td>
<td>3.8 (2.8 – 4.2)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1.7 (0.7 – 5.3)</td>
<td>1.3 (0.9 – 11.7)</td>
<td>1.9 (1.1 – 5.0)</td>
</tr>
<tr>
<td>P-selectin</td>
<td>6.0 (5.3 – 10.2)</td>
<td>7.3 (5.9 – 8.6)</td>
<td>5.8 (4.9 – 8.5)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>8.2 (4.9 – 11.5)</td>
<td>13.5 (5.4 – 19.8)</td>
<td>16.3 (6.1 – 24.3)</td>
</tr>
<tr>
<td>NGAL</td>
<td>9.9 (8.3 – 13.8)</td>
<td>14.4 (8.4 – 51.9)</td>
<td>13.2 (10.4 – 18.0)</td>
</tr>
<tr>
<td>ADAMTS-13</td>
<td>127.1 (98.3 – 165.7)</td>
<td>131.5 (118.2 – 141.7)</td>
<td>112.9 (95.0 – 144.9)</td>
</tr>
<tr>
<td>sAA</td>
<td>69.5 (11.7 – 158.5)</td>
<td>15.8 (12.7 – 90.3)</td>
<td>74.8 (10.3 – 113.2)</td>
</tr>
</tbody>
</table>

Data as median with interquartile range. MPO, myeloperoxidase; GDF-15, growth differentiation factor 15; sICAM-1, soluble intercellular adhesion molecule 1; NGAL, neutrophil gelatinase associated lipocalin; ADAMTS-13, A desintegrin and metalloprotease with thrombospondin type 1 repeats 13; sAA, serum amyloid A.
**Table S4. Correlations between concentration and activity of myeloperoxidase and clinical parameters from patients without antihypertensive medication**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Pregnancy</th>
<th>Gestational Hypertension</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Activity</td>
<td>Concentration</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.1 (0.1)</td>
<td>0.01 (0.2)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>Prepregnancy BMI (Kg/m²)</td>
<td>0.7 (0.04)</td>
<td>0.2 (0.1)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.5 (-0.1)</td>
<td>0.7 (-0.03)</td>
<td>0.03 (0.3)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.4 (-0.1)</td>
<td>0.3 (0.1)</td>
<td>0.04 (0.2)</td>
</tr>
<tr>
<td>GA at blood collection (weeks)</td>
<td>0.1 (-0.1)</td>
<td>0.9 (0.02)</td>
<td>1.0 (-0.00)</td>
</tr>
<tr>
<td>RBC (x10⁶/mL)</td>
<td>0.003 (0.4)*</td>
<td>0.5 (0.1)</td>
<td>0.4 (-0.1)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.01 (0.2)*</td>
<td>0.2 (0.1)</td>
<td>0.2 (-0.2)</td>
</tr>
<tr>
<td>WBC (x10³/mL)</td>
<td>0.4 (-0.1)</td>
<td>0.7 (-0.1)</td>
<td>0.3 (-0.1)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>0.8 (0.04)</td>
<td>0.2 (-0.2)</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td>Fasting glucose (mg/mL)</td>
<td>0.9 (0.01)</td>
<td>0.9 (-0.01)</td>
<td>0.1 (0.3)</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>ND</td>
<td>ND</td>
<td>0.7 (-0.1)</td>
</tr>
<tr>
<td>GA at delivery (weeks)</td>
<td>0.3 (0.1)</td>
<td>0.9 (-0.01)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>NBW (g)</td>
<td>0.9 (0.01)</td>
<td>0.7 (-0.03)</td>
<td>0.9 (-0.02)</td>
</tr>
</tbody>
</table>

Data as $P$ value (correlation coefficient). BMI represents body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GA, gestational age; RBC, red blood cells; WBC, white blood cells; ND, not determined; NBW, newborn weight. * Statistically significant ($P \leq 0.01$).
Table S5. Correlations between concentration and activity of myeloperoxidase and plasma nitrite from healthy pregnant, and gestational hypertensive and preeclampsia subgrouped in with (+) or without (-) antihypertensive treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Pregnancy</th>
<th>Gestational Hypertension</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Activity</td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Plasma nitrite (nM)</td>
<td>0.3 (-0.1)</td>
<td>0.7 (0.04)</td>
<td>0.9 (0.03)</td>
</tr>
</tbody>
</table>

Data as $P$ value (correlation coefficient). NO represents nitric oxide; +, patients with antihypertensive treatment; -, patients without antihypertensive treatment.
Table S6. Overview of studies that analyzed the relation of myeloperoxidase and preeclampsia

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Ref.</th>
<th>Sample type</th>
<th>n, PE(HP)</th>
<th>Gestational age (weeks)</th>
<th>Maternal age PE(HP)</th>
<th>MPO analysis type (method)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hung et al. (2012)</td>
<td>4</td>
<td>Heparin+EDTA-plasma*</td>
<td>20 (40)</td>
<td>38.3 (38.7) †</td>
<td>32.0 (31.8)</td>
<td>Concentration (ELISA)</td>
<td>No difference PE vs HP (P&gt;0.05)</td>
</tr>
<tr>
<td>Bowen et al. (2001)</td>
<td>5</td>
<td>Plasma/Serum*</td>
<td>21 (29)</td>
<td>37.8 (37.0) †</td>
<td>20.3 (19.4)</td>
<td>Concentration (ELISA)</td>
<td>No difference PE vs HP (P=0.35)</td>
</tr>
<tr>
<td>Karacay et al. (2010)</td>
<td>6</td>
<td>Serum</td>
<td>27 (29)</td>
<td>34 (29) at blood collection</td>
<td>26.0 (26.8)</td>
<td>Concentration (ELISA)</td>
<td>No difference PE vs HP (P=0.97)</td>
</tr>
<tr>
<td>Noyan et al. (2006)</td>
<td>7</td>
<td>Serum</td>
<td>21 (19)</td>
<td>34.0 (30.8) †</td>
<td>31.6 (27.6)</td>
<td>Activity (colorimetric)</td>
<td>Higher in PE vs HP (P&lt;0.004)</td>
</tr>
<tr>
<td>Gandley et al. (2008)</td>
<td>8</td>
<td>EDTA-plasma</td>
<td>11 (30)</td>
<td>35 (36) at blood collection</td>
<td>22 (27)</td>
<td>Concentration (ELISA)</td>
<td>Higher in PE vs HP (P=0.025)</td>
</tr>
<tr>
<td>Kordoglu et al. (2012)</td>
<td>9</td>
<td>EDTA-plasma</td>
<td>61 (50)</td>
<td>33.5 (38.7) †</td>
<td>29.7 (28.3)</td>
<td>Activity (colorimetric)</td>
<td>Higher in PE vs HP (P=0.005)</td>
</tr>
<tr>
<td>Mellembakken et al. (2001)</td>
<td>10</td>
<td>EDTA-plasma</td>
<td>15 (19)</td>
<td>34 (38) †</td>
<td>32 (33)</td>
<td>Concentration (ELISA)</td>
<td>Higher in PE vs HP (P&lt;0.01)</td>
</tr>
</tbody>
</table>

MPO represents myeloperoxidase; HP, healthy pregnant; PE, preeclampsia. *anticoagulant from collection tube not specified; † gestational age not specified.
Figure S1. Schematic diagram of the study workflow: multiplex assay led to selection of myeloperoxidase (MPO) as the main biomarker of the subsequent analysis. PE, preeclampsia; EDTA, ethylenediaminetetraacetic acid.
Figure S2. Boxplot of log concentration of biomarkers from cardiovascular disease panel in culture supernatant after incubation with 20% of plasma collected in heparin tubes from healthy pregnant women (white columns; n=25), gestational hypertensive (grey columns; n=10) and preeclamptic (black columns; n=26) women for 24 hours. Myeloperoxidase levels were significantly higher in preeclamptic than in healthy pregnant women group (P=0.02). Analytes were quantified by multiplex assay with Luminex xMAP® technology. Boxplot indicate median [min-max]. MPO represents myeloperoxidase; GDF-15, growth differentiation factor 15; Myo, myoglobin; P-sel, P-selectin; sICAM-1, soluble intercellular adhesion molecule 1; NGAL, neutrophil gelatinase associated lipocalin; ADAMTS-13, A desintegrin and metalloprotease with thrombospondin type 1 repeats 13; sAA, serum amyloid A. Comparison between groups were by ANOVA 1-way followed by Tukey’s post-test. *P<0.05 vs healthy pregnancy. Log transformation applied only for better visualization of the results.
Figure S3. MPO concentration and activity in plasma from EDTA tubes from healthy pregnant women (HP, white columns), gestational hypertensive (GH, grey columns) and preeclampsia (PE, black columns) patients without IUGR and subgrouped in patients on (+) and without (-) antihypertensive treatment. (A) MPO concentration in treated groups showed to be higher in HP (n=204, \( P=0.02 \)) than in GH (n=73) and PE (n=80). In groups without treatment, PE (n=31) showed higher levels than GH (n=32) and HP (n=204, \( P<0.0001 \)). (B) MPO activity showed no significant differences in treated groups (\( P=0.8 \)), however, comparing not treated groups, GH (n=32) had higher activity than HP (n=219) and PE (n=38, \( P=0.003 \)). Boxplot indicate median [min-max]. Comparison between groups was by Kruskal-Wallis followed by Dunn’s post-test. \*\( P<0.05 \) vs HP, †\( P<0.05 \) vs GH (-), ‡\( P<0.05 \) vs PE (-), §§\( P<0.05 \) vs PE (+).
Figure S4. Inhibition of plasma-EDTA enzymatic activity by 4-ABAH. Activity before (white columns) and after (black columns) incubation of plasma from healthy pregnant (n=104), gestational hypertensive (n=53) and preeclamptic (n=83) women with MPO inhibitor 4-ABAH (500μM / 30 minutes at 37°C). The presence of the inhibitor decreased plasma enzymatic activity in all groups (P<0.0001), indicating that activities found (Figure 2B) were mostly derived from myeloperoxidase. HP represents healthy pregnant; GH, gestational hypertension; PE, preeclampsia; (-), plasmatic activity without 4-ABAH addition; +, plasmatic activity after 4-ABAH addition. Data as mean±SEM. Comparisons between groups were by Mann-Whitney’s test. All P<0.05.
Figure S5. Correlations among healthy pregnant women between MPO concentration and red blood cells (A; $P=0.003$, $r=0.4$) and hemoglobin (B; $P=0.01$, $r=0.2$). RBC means red blood cell. Correlation assessed by Spearman’s test.
Figure S6. Comparison of MPO concentration and activity between plasma from EDTA tubes (white columns) and plasma from heparin tubes (black columns). (A) Concentration of MPO in HP (n=43), GH (n=34) and PE (n=37) plasma from heparin tubes were lower than from EDTA tubes ($P<0.0001$, $P=0.02$ and $P=0.04$, respectively). (B) MPO activity in plasma from heparin tubes from HP (n=23), GH (n=22) and PE (n=23) were lower than from EDTA tubes ($P<0.0001$). HP represents healthy pregnant; GH, gestational hypertension; PE, preeclampsia. Boxplot indicate median [min-max]. Comparison between plasma from heparin vs EDTA tubes in each group were by Mann-Whitney's test. *$P<0.05$ vs EDTA from respective group.
Figure S7. Plasma MPO activity inhibition by heparin. Enzymatic activity before (white columns) and after (black columns) incubation of plasma from healthy pregnant (HP, n=6), gestational hypertensive (GH, n=6) and preeclamptic (PE, n=6) women with heparin solution 14U/mL for 30 minutes. The anticoagulant addition significantly reduced the activity in all groups ($P=0.01$, $P=0.03$ and $P=0.0002$, respectively), which indicate its inhibitory capacity. (-) represents plasmatic activity without heparin; (+), plasmatic activity after incubation with heparin. Boxplot indicate median [min-max]. Comparisons between before and after heparin addition were by Mann-Whitney’s test. *$P<0.05$ vs – from respective group.