Heparins Increase Endothelial Nitric Oxide Bioavailability by Liberating Vessel-Immobilized Myeloperoxidase

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Background—Neutrophils and monocytes are centrally linked to vascular inflammatory disease, and leukocyte-derived myeloperoxidase (MPO) has emerged as an important mechanistic participant in impaired vasomotor function. MPO binds to and transcytoses endothelial cells in a glycosaminoglycan-dependent manner, and MPO binding to the vessel wall is a prerequisite for MPO-dependent oxidation of endothelium-derived nitric oxide (NO) and impairment of endothelial function in animal models. In the present study, we investigated whether heparin mobilizes MPO from vascular compartments in humans and defined whether this translates into increased vascular NO bioavailability and function.

Methods and Results—Plasma MPO levels before and after heparin administration were assessed by ELISA in 109 patients undergoing coronary angiography. Whereas baseline plasma MPO levels did not differ between patients with or without angiographically detectable coronary artery disease (CAD), the increase in MPO plasma content on bolus heparin administration was higher in patients with CAD (P<0.01). Heparin treatment also improved endothelial NO bioavailability, as evidenced by flow-mediated dilation (P<0.01) and by acetylcholine-induced changes in forearm blood flow (P<0.01). The extent of heparin-induced MPO release was correlated with improvement in endothelial function (r=0.69, P<0.01). Moreover, and consistent with this tenet, ex vivo heparin treatment of extracellular matrix proteins, cultured endothelial cells, and saphenous vein graft specimens from CAD patients decreased MPO burden.

Conclusions—Mobilization of vessel-associated MPO may represent an important mechanism by which heparins exert antiinflammatory effects and increase vascular NO bioavailability. These data add to the growing body of evidence for a causal role of MPO in compromised vascular NO signaling in humans. (Circulation. 2006;113:1871-1878.)

Key Words: atherosclerosis ■ coronary disease ■ endothelium ■ inflammation ■ leukocytes

Recruitment and activation of leukocytes, in particular, neutrophils and monocytes, is a central event in acute and chronic vascular inflammatory disease.1,2 One of the principal enzymes released from activated leukocytes is myeloperoxidase (MPO). This redox-activated hemoprotein is abundantly expressed in circulating neutrophils, monocytes, and tissue-associated macrophages and not only possesses potent bactericidal properties but also can be mechanistically linked to the underlying pathophysiology of chronic vascular inflammatory diseases, such as atherosclerosis and coronary artery disease (CAD).3,4 For example, MPO, located in the atherosclerotic lesion,5 mediates the oxidation of lipoproteins,6 catalyzes the nitration of tyrosine residues, and has been shown to deplete endothelium-derived nitric oxide (NO) through its use as a substrate and by the generation of small radical intermediates.7–12

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A principal prerequisite for the NO-oxidizing properties of MPO is its sequestration into the subendothelial space. Studies involving cultured endothelial cells and rat aortic rings have revealed that MPO binds to the cell surface and is transcytosed toward the subendothelial matrix in a heparin glycosaminoglycan (GAG)-dependent manner.10 So far, studies aiming to assess the relevance of MPO in mediating proinflammatory reactions in humans have been restricted to the analysis of circulating non–vessel-associated MPO.13–15 Thus, it remains unclear whether luminal (plasma or serum)
MPO levels reliably reflect MPO deposition within the vessel wall. Because the strategic localization of MPO in the subendothelial space has been demonstrated to be necessary for MPO to affect NO signaling pathways, the link between vessel wall-associated MPO and endothelial function in humans has remained elusive.

Because ex vivo studies have revealed that the binding of MPO to endothelial cells is prevented by heparins, we reasoned that systemic administration of heparins might release vessel wall–immobilized MPO in vivo. In the present study, we demonstrate that (1) patients with CAD, compared with control subjects, reveal an increased vascular deposition of MPO; (2) heparin administration improves endothelial NO bioavailability; and (3) the heparin-induced improvement of endothelial function is correlated with the extent of MPO liberation from the vessel wall into the luminal space.

Methods

Study Outline
The protocol was approved by the Hamburg medical board, and every patient gave written informed consent. One hundred nine consecutive patients who underwent coronary angiography at this institution and received a heparin bolus during intervention were included in the trial. CAD was defined by a history of myocardial infarction or coronary revascularization procedures or the presence of ≥50% luminal stenosis in at least 1 of the coronary arteries, as evidenced by coronary angiography. Patients with acute coronary syndromes within 1 month before study entry, those with congestive heart failure, those with impaired renal function (creatinine >2.0 mg/dL), and those with a history of long-acting antianginal medication were excluded. Blood samples were taken from each subject before and 15 minutes after administration of unfractionated heparin (70 U/kg body wt), and plasma was frozen at −80°C until further analysis.

With the use of venous occlusion plethysmography, 7 consecutive patients underwent assessment of acetylcholine (ACh)-induced changes in forearm blood flow in response to saline (NaCl), heparin, and Nω-monomethyl-L-arginine (L-NMMA). In addition, 27 subjects were randomized in a 2:1 fashion to either receive heparin (n=18) or NaCl (n=9), respectively, before and after the assessment of flow-mediated dilation (FMD), as described below.

Vascular Function Tests

Forearm Blood Flow in Response to ACh
To directly assess functional changes in vascular NO bioavailability and signaling, ACh-dependent increases in forearm blood flow were assessed by venous occlusion plethysmography, as described previously.16 In brief, a 20-gauge polyethylene catheter was inserted into the brachial artery. The strain gauge was connected to an electronically calibrated plethysmograph. A wrist cuff was inflated to 40 mm Hg to occlude venous outflow from the extremity. Flow measurements were recorded for 5 seconds every 10 seconds; the mean flow value of 7 consecutive readings was used for analyses. Basal measurements were obtained during intra-arterial infusion of 0.9% saline at a rate of 1.66 mL/min. Endothelium-derived vasodilation was assessed by infusing ACh in increasing concentrations of 7.5, 15, and 30 μg/min into the brachial artery. Sodium nitroprusside (1, 3, and 10 μg/min) was administered at 30 μg/min. To evaluate the NO-specific effects of changes in forearm blood flow, patients subsequently received intra-arterial infusions of the NO synthase inhibitor L-NMMA (16 μmol/min), and measurements were repeated. After intravenous administration of heparin (70 U/μg body wt), the protocol was repeated as above.

Determination of FMD
Ultrasound determination of FMD was performed according to the guidelines of the American College of Cardiology. Briefly, a Siemens (Iselin, NJ) Sonoline G50 ultrasound system with a 12-MHz linear array transducer was used to record 2-dimensional cine sequences of the brachial artery over 4 seconds at baseline and 1 minute after the induction of reactive hyperemia by 5-minute cuff occlusion of the forearm. The velocity time integral of Doppler flow was assessed by pulsed-wave Doppler with correction of insonation angle at baseline and peak hyperemic flow to calculate the flow ratio. Flow-independent vasodilation was assessed after determination of brachial artery diameter before and 4 minutes after the administration of nitroglycerine (0.4 mg). Subjects were instructed not to eat, drink, or smoke within 12 hours before testing. Measurements were carried out at baseline and 15 minutes after the intravenous administration of heparin (70 U/kg body wt) or placebo, respectively. The second measurement was performed after a waiting period of 3 hours after the administration of nitroglycerine. Two-dimensional sequences were analyzed by use of edge-detection software (Brachial Analyzer, Medical Imaging Applications LLC, Coralville, Iowa). The operators were blinded to patients’ treatments.

Determination of MPO Content in Matrix Proteins, Endothelial Cells, and Human Saphenous Veins
To assess the effect of heparin on the mobilization of MPO bound to extracellular matrices, fibronectin and collagen (13 μg/cm²), Sigma, Sigma-Aldrich, Inc, St Louis, Mo) were exposed to MPO (13 nmol/L, 2 hours at room temperature in phosphate-buffered saline), washed once, and incubated with heparin (20 U/mL, 20 minutes). Subsequently, supernatants were analyzed for MPO by ELISA, and MPO content was determined by Western blotting with the use of a polyclonal anti-MPO antibody (1:10,000, Calbiochem, EMD Biosciences, Inc, Merck KGaA, Darmstadt, Germany) and enhanced chemiluminescence for detection.

To determine the effect of heparin on the liberation of endotheli- um-bound MPO, cultured human umbilical vein endothelial cells (HUVECs) were grown to confluence, exposed to MPO (13 nmol/L, 2 hours; Planta Natural Products, Vienna, Austria), washed to remove nonadherent enzyme, and in some cases exposed to heparin (20 U/mL, 20 minutes). MPO content in the supernatant and cell lysates was determined by ELISA, as described below. MPO activity was assessed as described previously.16 To test the effect of heparin on MPO mobilization in human vessels, nonheparinized specimens from saphenous vein grafts from patients undergoing coronary artery bypass surgery were liberated from adventitial tissue. Vessels were divided into equal parts and exposed to either heparin (20 U/mL) or saline (0.9%) for 30 minutes at 37°C. Subsequently, tissue was homogenized as previously described, and proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.17 MPO protein content was determined by using a polyclonal anti-MPO antibody (1:10,000; Calbiochem) and enhanced chemiluminescence for detection.

Assessment of Elastase, MPO, and Triglyceride Plasma Levels
MPO and elastase plasma levels were determined by ELISA according to the manufacturer’s recommendations (Calbiochem and IBL Hamburg, Germany, respectively). All plasma samples were collected in heparinized tubes, with a final heparin concentration of 16 U/mL blood. In vitro supplementation of plasma samples with additional heparin (1 to 10 U/mL) did not affect MPO recovery by ELISA (data not shown). MPO recovery in heparinized plasma (as assessed by ex vivo addition of MPO to plasma from MPO-deficient individuals) was ~0.5%. After MPO plasma content (n=50) was analyzed by ELISA, recovering >95% of plasma MPO (Prognostix, Cleveland, Ohio), a high linear correlation was observed between the 2 ELISAs (r=0.78, P<0.001). Triglyceride levels were determined with a Hitachi (Tokyo, Japan) Modular Analyzer 0303 GS by an enzymatic method.
Statistical Analysis
Categorical data are presented as frequencies and percentages and were compared by χ² test and the Fisher exact test. Continuous variables were tested for normal distribution by use of the Kolmogorov-Smirnov test. Data with normal distribution are presented as mean±SD; non-normally distributed data are presented as median and interquartile range (IR). For normally distributed data, Student paired and unpaired t tests were used. One-way ANOVA for repeated measures using the Bonferroni method for multiple comparisons was used for venous plethysmographic data. Comparisons for nonnormally distributed data were performed by the Mann-Whitney U test and Wilcoxon signed rank sum test. For assessment of the association between FMD and MPO, the Pearson correlation was applied. Because CAD patients and control subjects showed significant differences in baseline characteristics, multivariate ANOVA accounting for CAD, age, gender, diabetes, and intake of lipid-lowering medication on MPO increase was performed. Because MPO levels revealed a non-normal distribution, a log-transformation of data was performed before testing. A value of \( P<0.05 \) was considered statistically significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the article as written.

Results
Effect of Heparin on Vessel Wall–Immobilized MPO
Heparins have been previously demonstrated to prevent the binding of MPO to cultured endothelial cells and isolated rat aortic ring segments.\(^8\),\(^10\) To test whether heparins also reverse MPO binding to the vessel wall, isolated extracellular matrix proteins, cultured endothelial cells, and saphenous vein graft specimens from patients undergoing aortocoronary bypass surgery were analyzed for MPO before and after treatment with heparin. MPO binding to fibronectin and collagen was markedly reversed in the presence of heparin (Figure 1A), as was MPO association with cultured HUVECs. Heparin treatment increased MPO content and MPO activity in the supernatant and lysate being affected accordingly (right). \( * P<0.05 \) (n=9). ANOVA with Tukey post hoc analysis was used to assess differences between groups.

Figure 1. Heparin releases MPO from matrix proteins, endothelial cells, and vein grafts in patients with CAD. A, Effect of heparin on MPO mobilization from extracellular matrices. Collagen and fibronectin were exposed to MPO and in some cases treated with heparin (see Methods for details). Heparin increased MPO levels in the supernatant, as assessed by ELISA (top), whereas matrix-adherent MPO was reduced, as revealed by Western blotting (bottom). \( * P<0.01 \). MPO hc indicates MPO heavy chain. B, Liberation of endothelial cell–adherent MPO by heparins. Confluent HUVECs were incubated with MPO and in some cases treated with heparin. Heparin increased MPO content in the supernatant and decreased MPO content in the cell lysates (left), with MPO activity in the supernatant and lysate being affected accordingly (right). \( * P<0.05 \). C, Saphenous vein graft specimens from patients undergoing aortocoronary bypass grafting treated with saline or heparin. Tissue homogenates were analyzed for MPO by the Western blotting technique. Heparin released MPO from the vessel. \( * P<0.05 \) (n=9). ANOVA with Tukey post hoc analysis was used to assess differences between groups.
TABLE 1. Clinical Characteristics of Patients in Whom MPO and Elastase Plasma Levels Were Assessed Before and After Intravenous Heparin Administration

<table>
<thead>
<tr>
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<th>CAD (n=78)</th>
<th>Non-CAD (n=31)</th>
<th>P</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>59 (75.6)</td>
<td>16 (51.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Female</td>
<td>19 (24.4)</td>
<td>15 (48.4)</td>
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</tr>
<tr>
<td>Age, y</td>
<td>66.4±8.5</td>
<td>58.9±10.1</td>
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<tr>
<td>Hypertension</td>
<td>70 (89.7)</td>
<td>16 (51.6)</td>
<td>0.01</td>
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<tr>
<td>Diabetes mellitus</td>
<td>27 (34.6)</td>
<td>3 (9.7)</td>
<td>0.01</td>
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<td>Smoker</td>
<td>25 (32.1)</td>
<td>4 (12.9)</td>
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<td>Family history</td>
<td>30 (38.5)</td>
<td>10 (32.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>LDL</td>
<td>111.9±39.0</td>
<td>133.0±40.3</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL</td>
<td>50.8±13.8</td>
<td>58.8±18.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>149.0±91.2</td>
<td>115.7±59.8</td>
<td>0.06</td>
</tr>
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<td>Statins</td>
<td>48 (61.5)</td>
<td>4 (12.9)</td>
<td>0.01</td>
</tr>
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<td>ACE inhibitor</td>
<td>38 (48.7)</td>
<td>11 (35.5)</td>
<td>0.28</td>
</tr>
<tr>
<td>AT1 receptor blocker</td>
<td>8 (10.3)</td>
<td>1 (3.2)</td>
<td>0.44</td>
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<tr>
<td>MPO, ng/mL</td>
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<tr>
<td>Before heparin</td>
<td>10.72 (7.89–13.10)</td>
<td>8.93 (7.66–10.95)</td>
<td>0.1</td>
</tr>
<tr>
<td>After heparin</td>
<td>17.06 (13.61–22.61)</td>
<td>13.57 (10.38–17.60)</td>
<td>0.03</td>
</tr>
<tr>
<td>Elastase, ng/mL</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before heparin</td>
<td>84.96 (54.12–144.62)</td>
<td>59.68 (41.33–108.37)</td>
<td>0.06</td>
</tr>
<tr>
<td>After heparin</td>
<td>80.86 (50.83–113.64)</td>
<td>54.76 (43.30–122.36)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Values are given as n (%), mean±SD, or median (interquartile range). ACE indicates angiotensin-converting enzyme; AT1, angiotensin II type 1.

MPO and Elastase Plasma Levels
Whereas baseline MPO plasma levels tended to be slightly higher in CAD patients (10.72 [IR 7.89 to 13.10] ng/mL in CAD patients versus 8.93 [IR 7.66 to 10.95] ng/mL in non-CAD subjects; P=0.1), both groups revealed a significant increase in MPO plasma levels on heparin administration (7.05 [IR 4.17 to 9.27], P<0.01 for CAD patients; 4.09 [IR 1.52 to 7.44], P<0.01 for control subjects). The increase in MPO levels after heparin administration was significantly higher in CAD patients compared with control subjects (P=0.01). Moreover, there was a significant difference in MPO increase after heparin administration between both groups (Figure 2). To exclude the possibility that the increase in MPO was due to increased degranulation of leukocytes, circulating levels of elastase (which is stored in the same granules in polymorphonuclear leukocytes as it is in MPO) were measured. Elastase plasma levels did not increase after heparin treatment but decreased in both groups (Figure 2). This finding suggests that increased MPO plasma levels in response to heparin do not reflect increased activation/degranulation of leukocytes and supports the view that the increase in circulating MPO reflects liberation of vessel wall–associated MPO.

MPO and NO Bioavailability
Given previous work identifying MPO as an enzyme capable of oxidizing endothelium-derived NO in vivo, a we tested the effect of vascular MPO liberation by heparin on vascular function in humans. Vascular function tests in patients were performed before and 15 minutes after the administration of heparin. ACh-induced changes in forearm blood flow, as assessed by venous occlusion plethysmography, were significantly increased after heparin injection, an effect that was completely inhibited in the presence of the NO synthase inhibitor L-NMMA (Figure 3). Endothelium-independent vasodilation in response to sodium nitroprusside was not altered in either group (not shown). The heparin-induced increase in vascular NO bioavailability and function was confirmed in conduit vessels; Forearm FMD in 18 patients (CAD, n=9; non-CAD, n=9) randomized to heparin was increased (from 4.50% to 9.15%, P<0.01), as opposed to those receiving NaCl (from 7.78±6.06% to 7.51±5.67%, P=0.86; Figure 4A). However, flow-independent dilation remained unchanged (Table 2). The increase in FMD after heparin administration was measured as a percentage and as absolute dilation and was observed in patients with and without CAD. The changes in FMD were significantly correlated with the changes in MPO plasma levels (r=0.69, P<0.01; Figure 4B and 4C, Table 3). Heparins also liberate endothelium-bound lipoprotein lipase, which increases triglyceride plasma levels and thus has an impact on the endothelial bioavailability of free fatty acids. Heparin treatment resulted in a significant decrease in triglyceride levels (for the heparin group, 90.13±33.39 mg/dL before versus 74.14±25.46 mg/dL after heparin administration; P=0.01). Partial correlation adjusting for changes in

Figure 2. Effect of heparin on circulating MPO and elastase levels in patients with and without CAD. Heparin bolus addition increased MPO plasma levels in patients with and without angiographically documented CAD; however, the increase in circulating MPO on heparinization (ΔMPO) was significantly greater in CAD patients compared with patients without CAD. In contrast, no difference in elastase plasma levels was observed in CAD patients compared with patients without CAD after heparin treatment. Center line indicates median; box and whiskers, IR and 2.5 and 97.5 percentiles, respectively. Mann-Whitney U test assessed differences between groups.

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MPO plasma content excluded a significant inverse correlation between changes in triglyceride levels and changes in FMD ($r=-0.37$, $P=0.08$), whereas the correlation between changes in MPO plasma levels and vasomotor function remained significant after adjusting for triglyceride plasma levels ($r=0.59$, $P<0.01$).

**Discussion**

The principal findings reported in the present study are as follows: (1) Heparin mobilizes MPO from vascular compartments, (2) heparin augments endothelial NO bioavailability and improves NO-dependent vascular relaxation in vivo, and

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**Figure 3.** Heparin increases endothelial NO bioavailability. ACh-induced increase in forearm blood flow as measured by venous occlusion plethysmography was significantly increased after administration of heparin (70 U/kg body wt) *$P<0.01$ versus NaCl treatment. L-NMMA inhibited the heparin-induced increase in forearm blood flow. Data are presented as mean±SEM. To assess differences between groups, ANOVA for repeated measures with the Bonferroni method for multiple comparisons was used.

**Figure 4.** Effect of heparin on FMD and MPO release. A, FMD in response to heparin is shown. Heparin treatment compared with NaCl treatment increased FMD. Minus sign indicates before treatment; plus sign, after treatment. B, The increase in FMD was accompanied by an increase in MPO plasma levels. C, The extent of MPO release on heparin treatment was correlated with the heparin-dependent increase in FMD ($r=0.69$, $P<0.01$). Values are presented as mean±SEM. Student paired and unpaired $t$ tests were used to assess differences between groups.
MPO, until recently solely viewed a bactericidal enzyme, has emerged as a critical mediator of chronic inflammatory diseases, such as atherosclerosis. MPO has been shown to oxidize high-density lipoproteins (HDLs) and LDLs and to activate metalloproteinases, thereby affecting the composition and vulnerability of the atherosclerotic plaque. MPO promotes tyrosine nitration, which has an adverse impact on the function of matrix proteins such as fibronectin, anti-inflammatory enzymes such as superoxide dismutase (SOD), and coagulation factors such as fibrinogen. Moreover, MPO exerts cytokine-like properties by modulating neutrophil activation states on binding to CD11b integrins. Of importance, MPO has also been shown to oxidize endothelium-derived NO, thereby modulating redox-sensitive signaling pathways in the vessel wall and its downstream effects on vascular reactivity. When all is considered, MPO stands out as a critical mediator affecting leukocyte, smooth muscle, and endothelial cell function. Oxidation of endothelium-derived NO by MPO has proven to be directly dependent on MPO sequestration into the subendothelial space via apical to basolateral transcytosis across the endothelium. Heparins have previously been shown to prevent binding of MPO to the endothelium in cell culture studies and isolated rat aortic vessel segments. The present study importantly adds to these findings, in that heparin not only prevented MPO binding to vascular compartments but also reversed vessel-immobilized MPO. That is, isolated matrix proteins, cultured endothelial cells, and entire vein grafts from patients with CAD revealed less MPO burden and MPO activity after exposure to heparin (Figure 1). This finding suggests deposition of MPO in a heparin-accessible compartment such as the subendothelial space, which is not only devoid of many of the competing substrates for MPO present in plasma but also particularly rich in low-molecular-weight substrates, which will enhance rather than inhibit MPO-driven NO catabolism.

Complementary to these observations, circulating plasma MPO levels in humans increased ∼1.6-fold after the administration of heparin (Figures 2 and 4B). Given the overall low recovery of MPO with the ELISA used, the extent of liberated MPO may be importantly underestimated. In contrast, plasma levels of elastase, a proteolytic enzyme, which is expressed in the same granules as MPO, did not increase on heparinization, further confirming that the elevation in MPO plasma levels reflects a release of vascular-bound MPO and is not a consequence of systemic neutrophil activation (Figure 2). Release of MPO from the vessel wall by heparin allowed for determination of the extent of vessel wall–immobilized MPO in stable CAD. The significantly higher MPO levels after heparin administration in CAD patients (Figure 2), despite nonsignificant differences in baseline plasma MPO levels between the CAD and non-CAD patients, further indicate that previous studies identifying MPO as a powerful marker of risk in CAD may even have underestimated the prognostic information obtained from this hemoprotein. The fact that baseline MPO plasma levels only tended to be higher in CAD patients suggests that (despite increased MPO burden and MPO activity per neutrophil in patients with stable CAD) activation and degranulation of MPO-containing leukocytes.
are not increased in patients with stable coronary disease but are restricted to patients with acute coronary syndromes.

Because MPO must be located within the vessel wall in order to interfere with NO-signaling cascades, the effect of heparin on NO-dependent vasomotor function was tested in conductance and resistance vessels of patients with and without CAD. Heparin improved microvascular function, yielding increased forearm blood flow in response to ACh, an effect that was NO specific, inasmuch as coinfusion with the NO synthase inhibitor L-NMMA blunted the effect of heparin (Figure 3). Also, heparin augmented flow in conductance vessels, as evidenced by the significant increase in FMD (Figure 4A). Inasmuch as endothelium-independent vasodilation remained unchanged, heparin may protect the endothelium from MPO-dependent NO oxidation, further supporting the pathophysiological significance of vessel-adherent MPO.

In light of increased vascular deposition of MPO in patients with CAD, the association between endothelial NO bioavailability and MPO may have been thus far undervalued.24,25

Heparins have previously been demonstrated to exert antiinflammatory effects in vascular disease that are irrespective of their anticoagulative properties. Potential antiinflammatory properties include decreased endothelial cell activation, inhibition of platelet activation, and binding to chemokines.26,27 Also, heparins are suggested to increase NO bioavailability in animal models of ischemia/reperfusion, sepsis, and balloon injury and in vessel segments from patients undergoing coronary artery bypass grafting; however, the underlying mechanism has remained elusive.28–31

Liberation of NO-oxidizing enzymes such as MPO, as reported in the present study, may be an important pathophysiological link by which heparins exert antiinflammatory effects. Besides MPO, xanthine oxidase (XO) has also been shown to associate with the endothelium in a GAG-dependent manner,32 and heparin treatment has been shown to result in increased circulating XO levels.33 However, whereas MPO is shown to bind to heparan sulfate–based GAGs, XO predominantly binds to chondroitin sulfate–containing GAGs on the endothelial cell surface.10,32 This suggests that heparin is more effective in releasing MPO from the vessel wall than heparin is in releasing XO; however, it is entirely possible that the 2 enzymes may function synergistically in consuming endothelium-derived NO. The ability of MPO to consume endothelium-derived NO is reinforced by the strong correlation between MPO release and improvement in endothelial function (Figure 4C), further supporting the tenet that MPO is a significant mediator of decreased vascular NO bioavailability under inflammatory conditions. The fact that changes in MPO plasma levels and improvement in endothelial function remained independent of changes in triglyceride levels further supports the tenet that the heparin-induced liberation of vessel-bound MPO reflects a critical mechanistic bond for the vasoactive properties of heparins.

Of significance, heparin also releases from the vessel wall extracellular SOD, an enzyme known to improve NO bioavailability by quenching levels of superoxide.34 However, the robust increase in NO-mediated flow after heparin administration as reported in the present study advocates that the release of vessel wall–immobilized NO oxidases such as MPO and XO overrides the loss of NO-preserving enzyme systems such as SOD. SOD provides the principal substrate of MPO by reduction of superoxide to hydrogen peroxide (H2O2), whereas superoxide is known to retard the oxidation capacity of MPO; thus, the removal of SOD and depletion of an H2O2-generating source may in fact be beneficial and represent an additional explanation for the net increase in NO bioavailability after exposure to heparins.

The present results need to be interpreted with caution because the reported mechanism for the NO-preserving effects of heparins does not exclude additional actions of heparin yielding increased vascular NO. However, given (1) the heparin-induced liberation of endothelial and matrix-bound MPO, (2) evidence of increased MPO deposition in the vasculature of patients with CAD, and (3) the strong correlation between heparin-induced MPO release and improvement in NO-dependent vascular relaxation, mobilization of vessel wall-bound MPO appears to be an important causal link accounting for improved vascular NO bioavailability after heparin administration.

In light of the prognostic implications of impaired endothelial NO bioavailability and in the absence of any specific inhibitor for MPO to date, strategies aiming to specifically remove vessel wall–immobilized NO oxidases may represent a potential adjunct treatment strategy in patients with inflammatory vascular disease.

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Disclosures

None.

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Myeloperoxidase (MPO), a heme enzyme abundantly expressed in neutrophils, monocytes, and macrophages, has long been solely viewed as an enzyme involved in host defense. However, recent data obtained in vitro and in animal models have revealed that MPO also modulates vascular tone by depleting the bioavailability of endothelium-derived nitric oxide (NO). A critical prerequisite for MPO to function in this regard is its binding to endothelial cells and its accumulation in the subendothelial space. In the present study, we show that heparin liberates vessel wall–immobilized MPO in humans. Compared with healthy control subjects, patients with stable coronary artery disease revealed increased vascular deposition of MPO, reflected by a higher degree of MPO mobilization after the administration of heparin. Heparin administration increased endothelial NO bioavailability in conductance and resistance vessels of patients with and without coronary disease. This heparin-dependent improvement in endothelial function was closely correlated with the extent of MPO liberation. In summary, these data underscore the significance of vessel wall–immobilized MPO for the regulation of endothelium-derived NO in patients with chronic inflammatory disease and point toward treatment strategies aiming to distract leukocyte-derived NO oxidases from the vascular bed.
Heparins Increase Endothelial Nitric Oxide Bioavailability by Liberating Vessel-Immobiled Myeloperoxidase
Stephan Baldus, Volker Rudolph, Mika Roiss, Wulf D. Ito, Tanja K. Rudolph, Jason P. Eiserich, Karsten Sydow, Denise Lau, Katalin Szöcs, Anna Klinke, Lukás Kubala, Lars Berglund, Sonja Schrepfer, Tobias Deuse, Munif Haddad, Tim Risius, Hanno Klemm, Hermann C. Reichenspurner, Thomas Meinertz and Thomas Heitzer

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