Interaction of myeloperoxidase with vascular NAD(P)H oxidase-derived reactive oxygen species in vasculature: implications for vascular diseases

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Zhang, Chunxiang, Jian Yang, Jonathan D. Jacobs, and Lisa K. Jennings. Interaction of myeloperoxidase with vascular NAD(P)H oxidase-derived reactive oxygen species in vasculature: implications for vascular diseases. Am J Physiol Heart Circ Physiol 285: H2563–H2572, 2003; 10.1152/ajpheart.00435.2003.—Vascular NAD(P)H oxidase-derived reactive oxygen species (ROS) such as hydrogen peroxide (H2O2) have emerged as important molecules in the pathogenesis of atherosclerosis, hypertension, and diabetic vascular complications. Additionally, myeloperoxidase (MPO), a transcytosisable heme protein that is derived from leukocytes, is also believed to play important roles in the above-mentioned inflammatory vascular diseases. Previous studies have shown that MPO-induced vascular injury responses are H2O2 dependent. It is well known that MPO can use leukocyte-derived H2O2; however, it is unknown whether the vascular-bound MPO can use vascular nonleukocyte oxidase-derived H2O2 to induce vascular injury. In the present study, ANG II was used to stimulate vascular NAD(P)H oxidases and increase their H2O2 production in the vascular wall, and vascular dysfunction was used as the vascular injury parameter. We demonstrated that vascular-bound MPO has sustained activity in the vasculature. MPO could use the vascular NAD(P)H oxidase-derived H2O2 to produce hypochlorous acid (HOCl) and its chlorinating species. More importantly, MPO derived HOCl and chlorinating species amplified the H2O2-induced vascular injury by additional impairment of endothelium-dependent relaxation. HOCl-modified low-density lipoprotein (LDL), a specific biomarker for the MPO-HOCI-chlorinating species pathway, was expressed in LDL and MPO-bound vessels with vascular nonleukocyte oxidase-derived H2O2. MPO-vascular NAD(P)H oxidase-HOCl-chlorinating species may represent a common pathogenic pathway in vascular diseases and a new mechanism involved in exacerbation of vascular diseases under inflammatory conditions.

endothelial dysfunction; atherosclerosis; inflammation; leukocyte

VASCULAR NAD(P)H oxidase-derived reactive oxygen species (ROS) such as superoxide (O2-) and hydrogen peroxide (H2O2) have emerged as important molecules in the pathogenesis of atherosclerosis (36, 38, 43), hypertension (30, 40), and diabetic vascular complications (14, 15).

Myeloperoxidase (MPO) is a heme protein derived from neutrophils, monocytes, and macrophages. It is well known that the defense of the organism through production of hypochlorous acid (HOCl) is the traditional role of MPO; however, there is a growing body of evidence suggesting that MPO might play important roles in noninfectious diseases, especially in the pathogenesis of the above-mentioned inflammatory vascular diseases (7, 33, 39). In this regard, MPO- and HOCl-modified low-density lipoprotein (LDL) are highly expressed in animal and human atherosclerotic vessels (19, 28, 29). Malle et al. (28) first found that HOCl-modified LDL existed in atherosclerotic plaques of rabbits in response to dietary cholesterol. MPO is the only enzyme known to generate HOCl in vivo, thus HOCl-modified LDL is a stable and specific biomarker of MPO-catalyzed oxidation in vivo. Colocalization of immunoreactive MPO- and HOCl-modified-LDL in serial sections of rabbit lesions provided convincing in vivo evidence for the MPO-H2O2-chloride system mediated oxidation of (lipo)proteins. The same group also found that MPO- and HOCl-modified LDL were highly expressed in human atherosclerotic lesions but not in normal control vessels (29). MPO and HOCl-modified LDL were located both in vascular cells and extracellular spaces (29). Furthermore, Hazen et al. (19) first found that 3-chlorotyrosine, another specific marker of MPO-catalyzed oxidation, was markedly elevated in LDL isolated from human atherosclerotic intima. In addition, one recent report showed that a significant positive correlation existed between the intima-to-media area ration (I/M) and MPO and HOCl-modified protein present in human atherosclerotic vessels (18). To further investigate the possible role of MPO in the development of atherosclerosis, two clinical studies were performed. One study showed that elevated levels of leukocyte and blood MPO are associated with the presence of coronary heart disease (47). Another study demonstrated that people with MPO deficiency had beneficial effects against cardiovascular damage (27).
In contrast to the above clinical studies, disruption of the MPO gene in LDL receptor-deficient mice resulted in increased atherosclerosis (6). The mouse and human difference is an important distinction with regard to the role of MPO in the pathogenesis of atherosclerosis (31). Normally, rodent leukocytes may not secrete active MPO. Indeed, there is no detectable MPO in the atherosclerotic vascular wall of LDL receptor-deficient mice (6).

Leukocyte activation leads to the release of MPO from storage granules into the extracellular space. Leukocyte NAD(P)H oxidase-derived $H_2O_2$ is the physiological substrate of MPO. MPO utilizes $H_2O_2$ to oxidize chloride, resulting in the formation of the proinflammatory oxidant HOCl and chlorinating species. Up to 40% of the $H_2O_2$ generated by activated leukocytes is used to form HOCl, and the local concentration of HOCl potentially exceeds 100 $\mu$/l under pathological conditions (3, 12, 22, 35). We (10, 44, 46) and other investigators (1, 23, 32) confirmed that there are at least three mechanisms involved in MPO-induced vascular injury responses: 1) consumption of nitric oxide (NO), 2) oxidation of LDL into the form that is taken up by macrophages in an uncontrolled manner, and 3) reaction with L-arginine and protein to produce endogenous NO synthesis inhibitors (Cl-L-Arg and HOCl-modified LDL). All three mechanisms are $H_2O_2$ dependent (4, 10, 19, 29, 32, 44, 46).

We further demonstrated that MPO is a transcytosesizable protein. Not only can infiltrated leukocytes release MPO into the vascular wall, but blood-derived MPO can also bind and infiltrate into the vascular wall directly (4, 10, 44). Our in vitro study showing the short-time (2 h) in vitro exposure to MPO (10–400 nM) resulted in a dose-dependent increase of vessel-bound MPO activity (0.054–2.967 U/g tissue) (44). This is comparable to the levels of MPO in vessels under pathological conditions such as sepsis and atherosclerosis (1.66 ± 0.43 U/g tissue and 0.53 ± 0.12 U/g tissue separately, our unpublished data). More interestingly, the vascular-bound MPO (0.054–2.967 U/g tissue) inhibited endothelium-dependent relaxation in a dose-dependent manner in the presence of $H_2O_2$ (44).

It is well known that MPO can use leukocyte NAD(P)H oxidase-derived $H_2O_2$; however, it is unknown whether MPO can use vascular NAD(P)H oxidase-derived $H_2O_2$ in the vascular wall. Our hypothesis is that vascular-bound MPO derived from both circulating blood and infiltrated phagocytes can use vascular NAD(P)H oxidase-derived $H_2O_2$ to produce HOCl and chlorinating species. This MPO-HOCl-chlorinating species pathway in the vascular wall plays an important role in vascular injury and development of vascular diseases.

**MATERIALS AND METHODS**

**Materials.** Human MPO, rabbit anti-human MPO, and polyclonal anti-HOCl-modified LDL antibody were purchased from Calbiochem. Mouse anti-rat CD45 (leukocyte common antigen, clone OX-1) was obtained from Pharmingen. 4-Aminobenzoic acid hydrazide (ABAH) was from Aldrich Chemical. Biotinylated anti-rabbit IgG antibody, Vectastain ABC kit, and DAB kit were from Vector Laboratories, and the osmotic minipump (ALZET model 2002) was obtained from Durect. All the other materials were from Sigma.

**Animals.** Ten-week-old male Sprague-Dawley rats (Harlan Breeding Laboratories; Indianapolis, IN) were used in the study. The animals were anesthetized with ketamine (60 kg/kg per xylazine 5 mg/kg). All protocols were approved by the Institutional Animal Care and Use Committee at the University of Tennessee and were consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1985).

**Vascular function assessments.** Isometric tension was measured in isolated aortic and carotid artery ring segments of control and experimental rats as described (45, 46). At the time the rat was euthanized, the rat thoracic aortas were excised and cleansed of fat and adhering tissue. The vessels were cut into individual ring segments (2–3 mm in width) and suspended from a force-displacement transducer in a tissue bath. Ring segments were bathed in Krebs-Henseleit (K-H) solution. The vessels were contracted to 50–60% of their maximal capacity (which is between 6 and 8 g for rat aorta) with phenylephrine (3 $\times$ 10$^{-9}$–10$^{-7}$ M). When tension development reached a plateau, ACH (10$^{-3}$–3 $\times$ 10$^{-8}$ M) was added cumulatively to the bath to stimulate endothelium-dependent relaxation. Endothelium-independent relaxation was tested by cumulative addition of the NO donor sodium nitroprusside (SNP).

To compare the vascular injury response caused by $H_2O_2$, HOCl, and Cl-L-Arg, the vessel segments were preincubated with a subtoxic dosage (5–50 μM) of these three compounds for 1 h, followed by extensive washing, and then endothelium-dependent and -independent relaxation were tested.

To test whether MPO utilizes vascular NAD(P)H oxidase-derived $H_2O_2$ as a substrate to produce HOCl and chlorinating species in the vascular wall, we incubated rat aortic ring segments with ANG II (100 nM) for 4 h to increase the activity of vascular NAD(P)H oxidases and the production of HOCl. Vessels incubated with vehicle were used as controls. After a 4-h incubation, the vessels were then incubated with MPO (400 nM) for another 1 h, followed by washing, and then the vascular function was determined. To determine whether MPO-induced vascular dysfunction is through the MPO-vascular NAD(P)H oxidase-HOCl-chlorinating species pathway, the vessels were coincubated with either $H_2O_2$ scavenger catalase (800 U/ml), HOCl scavenger L-methionine (100 μM), or anti-oxidant vitamin C (Vit C) (200 μM), which scavenges HOCl and reverses chlorinating species formation (8). Because L-methionine and Vit C may also scavenge other free radicals, MPO inhibitors, 5-fluorouracil (5-FU) (2 mg/ml) (2), and ABAH (100 μM) (24, 25) were coincubated with MPO to further test whether the MPO-induced injury response is via HOCl generation.

Heparin-binding superoxide dismutase (HB-SOD) prevents ROS, and ANG II induced vascular injury by catalyzing $O_2$ to HOCl, whereas the latter is catalyzed to $H_2O_2$ and $O_2$ by catalase. In the presence of MPO, if MPO uses $H_2O_2$ to produce HOCl and chlorinating species, the SOD-induced protective effect might attenuate. To test this, HB-SOD (20 U/ml) was added into ANG II-pretreated vessels with or without MPO, and vascular function was determined.

**Measurement of MPO activity.** Previously, we have shown that MPO can bind to the vascular wall in vitro (10, 44). To measure vessel-bound MPO activity over time, rat right internal carotid artery and the caudal origin of the common carotid artery (CA) were transiently clipped, and a polyethylene (PE-10) catheter was inserted from the right external...
carotid artery. The inside of the CA was flushed with 500 μl K-H buffer and then filled with 100 μl K-H buffer containing MPO (400 nM). After a 60-min incubation period, the vessel was flushed again, the external carotid artery was ligated, and then the CA blood flow was restored. The rats were euthanized at 0, 1, 3, 5, and 7 days after incubation, and MPO activity in CA was measured. Vessel MPO activity was determined as described (41). Rat carotid arteries were isolated, frozen in liquid N₂, and pulverized using a mortar and pestle. Samples were resuspended in 1 ml 50 mM potassium phosphate buffer (pH 6) containing 0.5% hexadecyltributyl phosphonium bromide. The sample was then homogenized for 3 min, followed by sonication. The suspension was centrifuged at 40,000 rpm for 15 min. An aliquot (0.1 ml) of the supernatant was added to 2.9 ml potassium phosphate buffer (pH 6) containing 0.53 mM o-dianisidine and 0.15 mM H₂O₂. Absorbance of the sample was monitored at 460 nm every 15 s for 5 min. Potassium phosphate buffer served as a blank. MPO activity was determined by dividing the absorbance change per minute by the molar extinction coefficient for o-dianisidine (ε = 1.13 × 10⁴ M⁻¹ cm⁻¹) and was normalized to protein concentration. One unit of MPO was defined as that of degrading 1 μmole peroxide per minute.

**Determination of vascular NAD(P)H oxidase activity and ROS production.** Vascular NAD(P)H oxidase activity and its product O₂⁻ were measured using lucigenin-derived chemiluminescence in vehicle-treated control and ANG II-treated (100 nM for 4 h) rat aorta segments (34). Briefly, after preparation, the vessel ring was placed in a K-H buffer and allowed to equilibrate for 30 min at 37°C. Scintillation vials containing 2 ml Krebs-HEPES buffer with 25 μM lucigenin were then placed into a scintillation counter switched to the out-of-coincidence mode. Lucigen counts were expressed as counts per minute per milligram dry weight. For NAD(P)H oxidase activity measurement, either NADH or NADPH was added to tissue homogenates. To determine the predominant source of ROS in the vessel wall in our model, vascular NAD(P)H oxidase inhibitors diphenylene iodonium (DPI, 10 μM) and apocynin (Apo, 0.3 mM), xanthine oxidases inhibitor allopurinol (10 μM), or NO synthase inhibitor N⁻nitro-L-arginine methyl ester (L-NAME) (100 μM) were added to the tissue bath 60 min before determination of O₂⁻ generation. H₂O₂ production and location was determined by transmission electron microscope without poststaining. Grids were examined and photographed at 75 kV in the out-of-coincidence mode.

**Immunohistochemistry.** To obtain further direct evidence that vessel-bound MPO can use vascular nonleukocyte oxidase-derived H₂O₂ to produce HOCl and chlorinating species in the vascular wall, we detected HOCl-modified LDL, a specific biomarker for the MPO-HOCl-chlorinating species pathway, by immunohistochemistry. The vessel segments from rat aortas were incubated with 1 mg/ml human native LDL and ANG II (100 nM) for 4 h to allow native LDL into the vascular wall and increase the production of vascular nonleukocyte oxidase-derived H₂O₂. The vessels were then incubated with MPO (400 nM) for another 1 h. Vessel segments without preincubation of any experimental reagents, preincubated with LDL alone, LDL, and ANG II but without MPO, MPO alone, or LDL and MPO without ANG II were also detected.

Vessels preincubated directly with HOCl-modified LDL (1 mg/ml), which was prepared by the reaction of human native LDL (1 mg/ml) and HOCl (1 mM), were used as positive controls. In addition, vessels incubated with MPO and exogenous H₂O₂ (10 μM) were also used as positive controls. All the vessel segments were washed extensively before fixation to remove any possible unbound protein and reagents. Artery ring segments were preserved in 10% buffered formalin and embedded in paraffin. HOCl-modified LDL immunostaining was performed in thin sections (5 μM) of tissue. Before incubation with the primary HOCl-modified LDL antibody (1:200 dilution for 30 min), tissue sections were treated with H₂O₂ to quench endogenous peroxidase activity. A biotinylated anti-rabbit IgG secondary antibody was then applied. Immunostaining was detected using a Vector ABC kit. Sections were counterstained with hematoxylin.

To confirm that the MPO activity in our time-course study was induced by vessel-bound MPO and to exclude any possible MPO activity induced by leukocyte accumulation, MPO and leukocyte immunostaining were performed in thin sections (5 μM) of rat right common carotid arteries 3 days after local short-time (1 h) incubation with 400 nM MPO. Before incubation with either the rabbit anti-human MPO antibody (1:1,500 dilution for 1 h) or mouse anti-rat CD45 (leukocyte common antigen, clone OX-1), tissue sections were treated with H₂O₂ to quench endogenous peroxidase activity. Either A biotinylated anti-rabbit IgG secondary antibody (for MPO staining) or A biotinylated anti-mouse IgG secondary antibody was then applied. Sections were treated with vehicle as vehicle controls, and sections of rat spleen were used as positive controls for CD45 staining. Immunostaining was detected using a Vector ABC kit. Sections were also counterstained with hematoxylin.

**RESULTS**

**MPO binds to and exhibits activity in the vascular wall for a significant period of time.** The results showed that MPO could bind to and exhibit activity in the vascular wall in vivo (Fig. 1). The activity of MPO in vessels after short-time (1 h) in vivo exposure to high concentration of human MPO (400 nM), followed by extensive washing was 1.61 ± 0.35 U/g tissue, which is the level in vessels under pathological conditions such as sepsis. After 24 h, the activity of vessel-bound MPO was reduced by 30%, and after 3 days, it was reduced by 59%. After 7 days, the MPO activity was reduced to the normal control level (Fig. 1). In sharp contrast, the untreated control vessels only have a little background activity without any change during the observed time course. Compared with that of leukocyte released H₂O₂ (4), we demonstrated that MPO activity has a long half-life (4 min vs. ~3 days, respectively) in the vascular wall.

To confirm that the sustained MPO activity is from the vascular-bound MPO but not from infiltrated leukocytes, both MPO and leukocyte immunostaining...
were performed in the carotid arteries 3 days after local MPO incubation. As shown in Fig. 2, the vascular-bound MPO was located in the intima, and, to a lesser extent, in the media near the intima. In contrast, no leukocyte CD45 immunostaining was observed in the MPO-treated sections, suggesting that the sustained MPO activity is induced by vascular-bound MPO but not by the infiltrated leukocytes.

MPO-derived HOCl and chlorinating species (Cl-L-Arg) impaired vascular function and were more potent than H₂O₂. To compare the effects of H₂O₂, HOCl, and Cl-L-Arg on vascular injury, we incubated vessels with the above three different agents (5–50 μM) for 1 h, followed by washing. Vascular function was then measured. We found H₂O₂ at 5 and 10 μM had no injury effect on the vascular function. H₂O₂ at 50 μM inhibited the endothelium-dependent relaxation only by 30.70%, whereas HOCl and Cl-L-Arg badly impaired the vascular function (88.66% and 97.69% inhibition of endothelium-dependent relaxation at 50 μM, respectively) (Fig. 3A). The results indicated that MPO-derived HOCl and chlorinated species amplify H₂O₂-induced vascular injury. In contrast, all three compounds had no inhibitory effects on the endothelium-independent relaxation to SNP at the experimental concentrations (Fig. 3B), suggesting the endothelium is the target of the MPO-induced acute injury response.

Interaction between MPO and vascular NAD(P)H oxidase-derived reactive H₂O₂ within the vascular wall.
H2O2, hypochlorous acid (HOCl), and Cl-L-Arg on vascular function. Vessel segments were put into tissue baths and incubated with 5, 10, and 50 μM H2O2, HOCl, or Cl-L-Arg for 1 h followed by washing with Krebs-Henseleit (K-H) buffer. In phenylephrine-contracted aorta ring segments, the endothelium-dependent relaxation was tested by addition of ACh (10⁻⁹.3 × 10⁻⁶ M), and the endothelium-independent relaxation was tested by addition of sodium nitroprusside (SNP) (10⁻⁹.3 × 10⁻⁶ M). Data are means ± SE. *P < 0.01 and **P < 0.001 compared with corresponding dose of H2O2.

To test whether MPO utilizes vascular NAD(P)H oxidase-derived H2O2 as a substrate to produce HOCl and chlorinating species in the vascular wall, we incubated rat aortic ring segments with ANG II (100 nM) for 4 h to increase the activity of vascular NAD(P)H oxidases and the production of H2O2. Our results showed that after incubation with ANG II for 4 h, vascular NAD(P)H oxidase activity and its products superoxide and H2O2 were increased (Fig. 4, A–C). There are three potential sources of superoxide and H2O2 in the vascular wall without leukocytes: vascular NAD(P)H oxidase, xanthine oxidase, and NO synthase. Our results indicate that vascular NAD(P)H oxidase is the predominant source of ROS in ANG II-treated vessels in vitro because xanthine oxidases inhibitor allopurinol (10 μM) or NO synthase inhibitor l-NAME (100 μM) had no significant effect on the increased production of superoxide, whereas vascular NAD(P)H oxidase inhibitors DPI (10 μM) or Apo (0.3 mM) reduced superoxide significantly (Fig. 4B). This suggests that vascular NAD(P)H oxidases is the main source of H2O2 in the vessel wall in our model. The result is consistent with one previous study showing that vascular NAD(P)H oxidase is the predominant source of ROS in vessels from ANG II-induced hypertensive animals (34).

After a 4-h incubation period with ANG II, the vessels were then incubated with MPO (400 nM) for another 1 h, followed by washing, and then vascular function was determined. The results showed that ANG II partially impaired the endothelium-dependent relaxation by a mechanism of increased ROS formation (Fig. 5A). MPO had no notable effect on control vessels; however, MPO significantly amplified the impairment effect on endothelium-dependent relaxation in ANG II pretreatment vessels (Fig. 5A). In contrast, ANG II and MPO had no injury effect on the endothelium-independent relaxation to SNP (Fig. 5B). The mechanism by which MPO induced the additional injury on endothelium-dependent relaxation is by the reaction with vascular NAD(P)H oxidase-derived H2O2 and its chlorinating species because the H2O2 scavenger catalase, the HOCl scavenger l-methionine, and antioxidant Vit C, which can reverse the formation of chlorinating species, can prevent the injury response (Fig. 5C). Also, coincubation with MPO inhibitors ABAH (100 μM) or 5-fluorouracil (2 mg/ml) blocked MPO-induced impairment of vascular function (Fig. 5D). Furthermore, although coincubation with HB-SOD prevented ANG II-induced endothelial dysfunction, the protective effect of HB-SOD disappeared in MPO-treated vessels (Fig. 5E). The results suggest that MPO uses vascular NAD(P)H oxidase-derived H2O2 to amplify the injury response.

**HOCl modified-LDL was expressed in the LDL-preincubated vascular wall containing both MPO and vascular NAD(P)H oxidases-derived H2O2.** Our results showed that there is no HOCl-modified LDL staining in the negative control (without primary antibody) (Fig. 6A), normal untreated segment (Fig. 6B), as well as in vessels incubated with LDL alone (Fig. 6Cc), ANG II and LDL (Fig. 6D), MPO alone (Fig. 6E), and LDL together with MPO (Fig. 6F). In sharp contrast, the vessels treated with LDL, ANG II, and MPO, which had LDL, vascular NAD(P)H oxidase-derived H2O2, and MPO in the vascular wall, had strong immunostaining (Fig. 6G). The HOCl-modified LDL was located in the intima and, to a lesser extent, in the media (Fig. 6G), which was similar to those in two positive controls (Fig. 6, H and I).

**DISCUSSION**

There is growing evidence of the significance of MPO in cardiovascular diseases, which includes atherosclerosis, ischemia-reperfusion injury, sepsis, and diabetic vascular complications. Our previous studies (10, 45, 46) have demonstrated that one of the important mechanisms involved in MPO-induced vascular injury is the reduction of NO bioavailability. We also found MPO is a transcytosable protein that can bind and infiltrate into the vascular wall (4, 10, 44). Not only can the
vascular-bound MPO localize in the extracellular space, but it can also enter vascular cells (10, 42). In the present study, we further demonstrated that the vascular-bound MPO has a relatively long half-life; it can remain in the vasculature for at least 3–5 days. This interesting result points to the importance of understanding the interaction of vascular-bound MPO, both from vascular-infiltrated activated phagocytes and from circulating blood, with vascular ROS such as H$_2$O$_2$, which has a very short half-life.

Our previous studies have shown that MPO-induced vascular injury responses are NAD(P)H oxidase-derived H$_2$O$_2$ dependent (10, 44–46). There are two different kinds of NAD(P)H oxidases: leukocyte-derived and nonleukocyte-derived NAD(P)H oxidase (13, 37). Vascular NAD(P)H oxidases belong to the nonleukocyte-derived NAD(P)H oxidase and are the major sources of ROS in both atherosclerotic and diabetic vasculature (13, 16, 20, 21, 43). Vascular NAD(P)H oxidases-derived ROS such as H$_2$O$_2$ have emerged as important molecules in pathogenesis of inflammatory vascular diseases. Recent reports show that the vascular NAD(P)H oxidases are structurally and genetically distinct from the leukocyte-derived NAD(P)H oxidase (9, 42). In contrast to leukocyte-derived NAD(P)H oxidase, which can only be activated and produce ROS in a respiratory burst manner, vascular NAD(P)H oxidases are low-output, slow-release enzymes and have a moderate constitutive activity, which is absent in phagocytes. In normal vessels, vascular NAD(P)H oxidase-derived H$_2$O$_2$ is catabolized to H$_2$O by catalase and loses any injury effects. However, under pathological conditions such as atherosclerosis, hypertension, and diabetes, the production of H$_2$O$_2$ is much higher than its degradation. Thus the H$_2$O$_2$ content in the vascular wall under the above-mentioned pathological conditions is much higher than in normal vessels, although the absolute tissue concentration needs to be determined (13, 16, 20, 21). One recent report (26) indicates that vascular NAD(P)H oxidase-derived ROS...
might be more important than leukocyte NAD(P)H oxidase-derived ROS in the pathogenesis of atherosclerosis, because a deficiency in leukocyte NAD(P)H oxidase fails to inhibit atherosclerosis in mice with either diet-induced or genetic forms of hypercholesterolemia. Although it is well known that MPO can use phagocyte-derived H$_2$O$_2$, there is a lack of direct evidence showing that the vascular-bound MPO can use vascular NAD(P)H oxidase-derived H$_2$O$_2$ to induce injury in the vascular wall.

In the current study, we used ANG II to increase vascular NAD(P)H oxidase and subsequent H$_2$O$_2$ production and vascular dysfunction as a vascular injury parameter. We confirmed that vascular NAD(P)H oxidase is the main source of the increased H$_2$O$_2$ in our vessel model system, which is consistent with previous studies (34). MPO can use the vascular NAD(P)H oxidase-derived H$_2$O$_2$ to amplify the ROS-induced vascular injury as showed by the further impairment of endothelium-dependent relaxation. The mechanism involved in MPO-induced vascular injury is by its reaction with H$_2$O$_2$ to produce HOCl and its chlorinating species. MPO inhibitors, H$_2$O$_2$ scavenger, HOCl scavenger, and antioxidant Vit C can prevent MPO-induced injury response. To further confirm the mechanism, we also used the SOD, which can protect the ROS-induced injury by the following protective pathway: NAD(P)H oxidase-superoxide-SOD-H$_2$O$_2$-catalase-H$_2$O$_2$. Indeed, our results showed that SOD protected against ANG II-induced vascular injury. In the presence of MPO, MPO will compete with catalase in catalyzing their common substrate H$_2$O$_2$ to produce HOCl and its chlorinating species.
chlorinating species, and the protective effect of SOD should be reduced. One recent report (5) also showed that in lipopolysaccharide-injected animals, which have MPO and increased O2/H2O2 production in the vascular wall, SOD failed to improve ACh-induced relaxation. Our results showed that the protective effect of SOD disappeared in MPO-bound vessels treated with ANG II. Finally, to obtain the direct evidence that vessel-bound MPO can use vascular NAD(P)H oxidase-derived H2O2 to produce HOCl and chlorinating species, we detected HOCl-modified LDL, a specific biomarker for MPO-HOCl-chlorinating species pathway, by immunohistochemistry in the vascular wall (17, 29). Our results showed that HOCl-modified LDL was located in the intima and, to a lesser extent, in the media.

It has been well known for a long time that systemic infection is one of the main inducing factors for the attack of coronary artery disease and stroke, and chronic systemic inflammation exacerbates the atherosclerotic lesions, but the molecular mechanism is still unclear. Under the above inflammatory conditions, the amount of MPO in circulating blood is increased and so is the MPO in the vascular wall. Our results in the present study indicate that MPO may be the main link between systemic inflammation and the exacerbation of vascular diseases.

In summary, vascular-bound MPO could use the vascular NAD(P)H oxidase-derived H2O2 to produce HOCl and its chlorinating species and amplify vascular NAD(P)H oxidase-derived ROS-induced injury in the vascular wall. NAD(P)H oxidase-derived H2O2 is increased in many inflammatory vascular diseases, and leukocyte activation, adhesion and infiltration into the vascular wall are the common events under these conditions. Furthermore, MPO- and HOCl-modified proteins are highly expressed in human atherosclerotic lesions. Thus MPO-HOCl-chlorinating species may represent a common pathogenic pathway in diverse inflammatory vascular diseases and a new mechanism in phagocyte and systemic infection-induced exacerbation of vascular diseases. Blocking the MPO-HOCl-
clorinating species pathway might be a new therapeutic approach to vascular diseases.

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DISCLOSURES

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