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EDITORIAL COMMENT

Can Myeloperoxidase Identify High-Risk Plaques and Subjects Harboring Them?*

Rika Kawakami, MD,[a] Aloke V. Finn, MD,[a,b] Renu Virmani, MD[a]

Although many researchers would say atherosclerosis is an inflammatory disease, most consider it a disease more directly related to lipid disorders. Based upon copious experimental data, it is irrefutable that inflammation enhances the progression of atherosclerosis. Perhaps the most notable attempt to demonstrate a critical role for inflammation in atherosclerosis was the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) which randomized high-risk subjects to 1 of 3 doses of the anti-inflammatory drug, canakinumab. Canakinumab is an antibody that targets the interleukin-1β innate immune pathway, a cytokine that is central to an inflammatory response that drives interleukin 6; the drug had already been approved for use in patients with rheumatologic disorders. In this trial, only the 150 mg dose showed a significantly lower rate of recurrent cardiovascular events vs the placebo,[1] and its use was associated with a higher incidence of fatal infection vs the placebo.[1]

Myeloperoxidase (MPO) is another pro-inflammatory enzyme that catalyzes the formation of several reactive oxygen species and has been found within atherosclerotic plaques. MPO is found in the granules of neutrophils and plays a specific role in bacteria death via the release of hydrogen peroxide.[2,3] MPO is expressed in myeloid lineages in all stages of maturation.[4] The intensity of MPO in monocytes is much weaker than in granulocytes.[5] The role of MPO in atherosclerosis is thought to occur through the generation of highly reactive hypochlorous acid, chloramines, tyrosyl radical, and so on, resulting in the oxidation of lipoproteins.[6] These modified lipoproteins are then taken up via the scavenger receptors present on macrophages which convert them into foamy (lipid-laden) macrophages, resulting in the progression of atherosclerosis.[4] Early studies showed that human macrophages in atherosclerotic plaques contain MPO.[7] This was followed by clinical studies that showed higher systemic levels of MPO, which predicted the subsequent cardiovascular event and the prognosis in patients with acute coronary syndrome.[8] Autopsy studies reported that unstable plaques, such as plaque rupture, healed ruptures, and thin cap fibroatheromas, contained higher levels of MPO than stable plaques.[9,10] Clinical studies in acute myocardial infarction patients showed higher circulating MPO levels in patients with plaque erosion vs rupture.[11] Similarly, in the ICARAS (Inflammation and Carotid Artery Risk for Atherosclerosis Study), high serum levels of MPO were associated with the progression of carotid atherosclerosis in patients with low levels of high-density lipoprotein cholesterol for a mean of 7.5 months during follow-up.[12] Although these data appear promising, further work is likely needed to develop MPO into a legitimate diagnostic and therapeutic target for the detection of high-risk plaque.

In this issue of *JACC: Advances*, Nadel et al[13] provides a further understanding of the role of MPO in atherosclerosis progression. They report on carotid endarterectomy specimens from 31 patients and coronary plaques removed from 12 patients undergoing cardiac transplantation. The authors show that intraplaque MPO activity, as detected by the conversion of hydroethidine to MPO-specific adduct 2-chloroethidium, is higher in unstable carotid and coronary plaques than in stable plaques.[13] Moreover, intraplaque MPO activity correlates with symptomatic

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From the [a]Department of Cardiovascular Pathology, CVPath Institute, Gaithersburg, Maryland, USA; and the [b]School of Medicine, University of Maryland, Baltimore, Maryland, USA.

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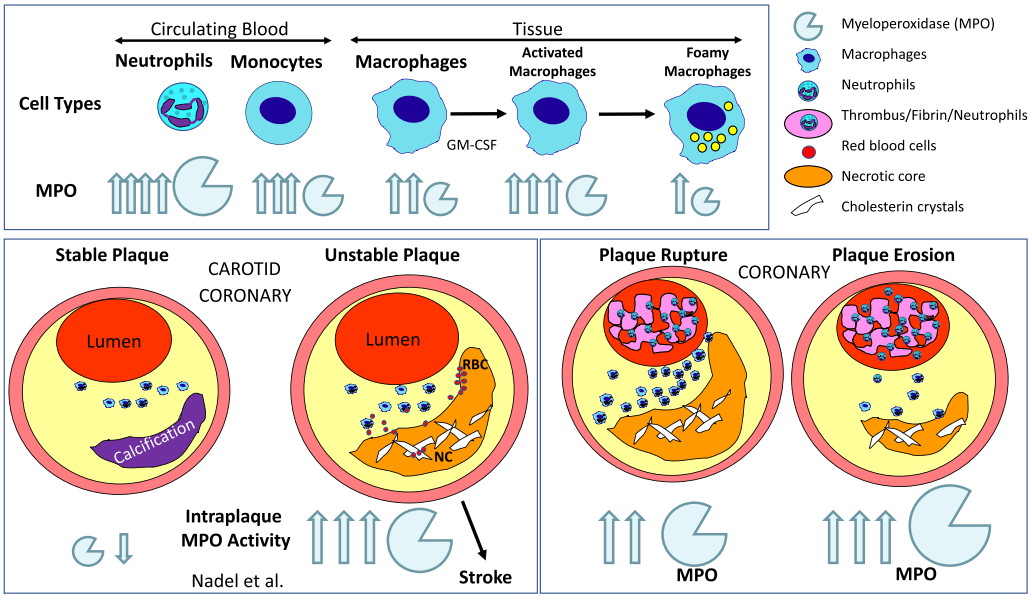
FIGURE 1 Myeloperoxidase in Blood and Plaques



MPO is primarily found in granules of neutrophils. Circulating monocytes also contain myeloperoxidase but less than neutrophils. Nadel et al[13] showed that unstable plaques had greater intraplaque myeloperoxidase activity than stables plaques, and myeloperoxidase activity was related to individuals presenting with symptoms or stroke. In atherosclerotic plaques, it is the unstable plaques that are the main source of myeloperoxidase and neutrophils reside not in the plaques but in the thrombus. Previous studies demonstrated that plaque erosion contains a higher cell density of myeloperoxidase-positive cells compared to plaque rupture, and these correlate with circulating myeloperoxidase levels. GM-CSF = granulocyte-macrophage colony-stimulating factor; NC = necrotic core; RBC = red blood cells.

carotid artery disease and stroke.[13] Overall, the correlation was better when utilizing histology as compared to the gross identification of unstable plaque. Intraplaque MPO activity was assessed by first homogenizing and standardized to protein concentration prior to undergoing MPO activity assay by liquid-chromatography mass spectrometry (MPO-specific chlorination of the synthetic compound hydroethidine to 2-chloroethidium). One surprising finding was the lack of correlation between serum levels of MPO and intraplaque MPO activity, although unstable plaques did have higher MPO activity than stable ones. However, computed tomography-derived determinants of unstable plaques did not reliably detect plaques that were MPO-rich. The authors never correlated the serum levels or MPO activity with the MPO-positive area within the same plaques or with the macrophage area derived from immunohistochemistry.

MPO is mostly derived from neutrophils, which are cells that are not usually present in plaques but are seen mostly within plaque-related thrombi.[11] The most frequent inflammatory cells in carotid and coronary plaques are macrophage foam cells derived from circulating monocytes. The main source of MPO in the circulation comes from circulating neutrophils, and MPO is stored in azurophilic granules (**Figure 1**). MPO can be released into the extracellular space via degranulation. Circulating monocytes have 1/3 the content of MPO as compared to neutrophils.[4] The differentiation of monocytes to macrophages is associated with the loss of MPO.[5]

There are some important limitations to the work of Nadel et al,[13] worth noting. The authors did not show quantitative data on cluster of differentiation 68-positive cells in stable or unstable plaques, nor did they show that MPO staining was seen in macrophages or any other cell types. These types of data would further substantiate their claim that MPO may be directly involved in plaque progression by showing how cells within the plaque produce MPO. Moreover, the lack of correlation between MPO activity in plaque and circulating serum MPO, a much more practical method to detect MPO in living subjects, raises further questions about how the 2 relate to each other and may perhaps have more to do with the absence of thrombi in the plaques collected. A prior study reported that patients with ST-segment elevation and non-ST-segment elevation acute myocardial infarction had very high levels of circulating MPO with a median of 600 to 800 ng/mL.[11] Whereas in the current

study, serum MPO levels were only 220 ± 114 ng/mL.[13] The lack of correlation may be due to only 18/30 carotid plaques having low attenuation without mentioning the presence or absence of a thrombus. Therefore, it is not surprising that the authors could not correlate either computed tomography-identified high vs low attenuation plaques, or the presence or absence of perivascular adipose tissue with MPO activity.[13]

A previous paper by the same authors reported on a tandem stenosis model that developed atherosclerotic plaque instability in Apoe −/− mice.[14] The authors nicely showed in Apoe −/− mice that MPO activity was 2-fold greater in unstable vs stable plaque phenotypes.[14] In a double knock-out with Apoe−/− Mpo−/− mice, the fibrous cap thickness increased more than in Apoe−/− mice, and there was also a decrease in plaque fibrin and hemosiderin content. The authors also showed that MPO-targeted gadolinium using a T1-weighted magnetic resonance sequence was able to discriminate between unstable and stable plaques. Unstable plaques showed greater MPO activity (2-chloroethidium in Apoe−/− mice undergoing tandem stenosis). Along these lines, the authors also showed that a 2-thioxanthine MPO inhibitor (AZM198) inhibited the activity of MPO in mice.[14] This leads to the question of why, in the current manuscript by Nadel et al,[13] findings were not fully recapitulated in humans? We believe the selection of human plaques may have been a major but possibly unavoidable limitation. The authors likely needed the presence of thrombus, which also contains high levels of MPO, and which were likely not present in the carotid endarterectomy samples (because thrombus is embolized due to high blood flow). The coronary arteries were obtained from hearts removed during transplantation and thus unlikely to have thrombi. Despite these limitations, the work of Nadel et al[13] adds important new insight into our knowledge of the role of MPO in human plaques and how it continues to further its development as a diagnostic and therapeutic target for the treatment of atherosclerosis.

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ADDRESS FOR CORRESPONDENCE: Dr Renu Virmani, 19 Firstfield Road, CVPath Institute, Inc, Gaithersburg, Maryland 20878, USA. E-mail: rvirmani@cvpath.org.

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