Targeting myeloperoxidase in inflammatory atherosclerosis

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This editorial refers to ‘Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque’[†], by I. Rashid et al., on page 3301.

Atherosclerosis is a chronic immune disorder driven by the accumulation of lipids in the vessel wall, resulting in the formation of plaques.¹ Progression of the disease may ultimately lead to peripheral artery disease or the occurrence of clinical events, including stroke and myocardial infarction. Our view on the atherosclerosis pathophysiology is shifting from an emphasis on lipids to vascular immunology, particularly macrophage dynamics. The recently completed CANTOS trial unveiled a decrease in recurrent cardiovascular events through treatment with an interleukin-1 β -blocking antibody as compared with placebo.² Cumulative evidence supports the notion that macrophage inflammation is an important driver of disease progression with major involvement of the haematopoietic organs. Myeloid cells produced by these tissues are recruited by endothelial cells and enabled entry into the plaque.³ Monocyte recruitment is an important driver of atherosclerosis in early stages, while in progressed plaques macrophage proliferation seems to dominate vessel wall inflammation.³ Neutrophils are the most abundant myeloid cells present in the circulation.⁴ Apart from the release of neutrophil extracellular traps (NETs), neutrophils secrete granulocytes to aid in degradation of microbiomes and to regulate immune responses.^{5,6} Their cargo is discharged upon activation in the plasma or when interaction with microbiomes is initiated.⁴ One of the most important enzymes released by neutrophils through degranulation is myeloperoxidase (MPO). In patients with elevated levels of circulating MPO, increased cardiovascular events are observed⁷ while, in atherosclerotic lesions, MPO interacts with the extracellular matrix to form hypochlorous acid by converting chloride anion and hydrogen peroxide.⁸ The presence of MPO in plaques has been associated with endothelium apoptosis, superficial erosion, and lesion rupture (*Take home figure*).^{6,9} Altogether, it is becoming increasingly evident that

MPO is a compelling imaging^{10,11} and therapeutic target^{12,13} in atherosclerosis.

In this issue of the *European Heart Journal*, using a mouse model of atherosclerosis, Rashid et al. describe the evaluation of MPO magnetic resonance imaging (MRI) to detect unstable plaques.¹⁴ Additionally, the study explores MPO (*Take home figure*) as a target for directed therapy aimed at stabilizing the plaque phenotype. Mice with an *Apoe*^{-/-} genetic background, an established atherosclerosis model, underwent tandem stenosis after being fed a 6-week-long enriched Western diet. The surgical procedure involves partially ligating the right common carotid artery at two places distally from the innominate artery. If successful, this results in the formation of plaques that display several characteristics observed in unstable human plaques, including intraplaque haemorrhage, disruptive fibrous caps, intraluminal stenosis, and neovessels.¹⁵

First, the authors compare a MPO-targeted gadolinium (MPO-Gd) contrast agent with the generic Gd-DTPA (Magnevist). MPO-Gd was observed to accumulate and be retained in plaques up to 60 min after injection, while Gd-DTPA washed out more rapidly. Importantly, MPO-Gd administration allowed distinguishing stable from unstable plaques by using a T1-weighted MR sequence resulting in a hyperintense vessel wall. Validation studies confirmed the *in vivo* findings by establishing surrogate markers of MPO activity using liquid chromatography tandem mass spectrometry (LC-MS/MS).¹⁶ To corroborate the agent's specificity, MPO was knocked-out in *Apoe*^{-/-} mice. The *Apoe*^{-/-} MPO^{-/-} double knockouts underwent tandem stenosis and were subjected to *in vivo* MRI after injection with MPO-Gd. Significantly less contrast was found compared with regular tandem stenosis *Apoe*^{-/-} mice. Immunohistochemistry with picosirius red staining revealed a significantly increased fibrous cap thickness and cap to lesion height ratio in the *Apoe*^{-/-} MPO^{-/-} double knockouts.

Rashid et al. subsequently studied whether blocking MPO in tandem stenosis *Apoe*^{-/-} mice through pharmacological inhibition with AZM198 showed similar findings compared with genetic deletion.

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Take home figure Top: myeloperoxidase-gadolinium (MPO-Gd) facilitates identification of unstable plaques in tandem stenosis *Apoe*^{-/-} mice with magnetic resonance imaging (MRI). Bottom: myeloperoxidase inhibition with AZM198 treatment results in formation of a more stable plaque phenotype with increased fibrous cap thickness, similar to the phenotype observed in tandem stenosis *Apoe*^{-/-} *MPO*^{-/-} double knockouts. Figures were made using Servier Medical Art (www.servier.com).

Indeed, they found the MPO-Gd contrast and LC-MS/MS measurements to be very similar to the values observed in the *Apoe*^{-/-} *MPO*^{-/-} double knockout mice. However, MPO staining showed no difference in MPO expression compared with non-treated tandem stenosis *Apoe*^{-/-} mice, possibly indicating that the activity rather than the availability of MPO is responsible for the effects. To investigate possible systemic adverse effects of the pharmacological inhibition, blood assays and extensive immunohistochemistry evaluation were performed. No alterations in circulating lipid or inflammatory cell levels were found. Immunohistochemistry unveiled no difference in haemoglobin expression; however, a clear difference was observed for the phagocytized remnants of haemoglobin, haemosiderin. Finally, reduced fibrin depositions were found in the MPO knockout and AZM198-treated mice compared with the regular tandem stenosis *Apoe*^{-/-} mice. The authors conclude that these results are indicative for reduced intraplaque haemorrhage. Together with the picrosirius red staining, these results suggest that an AZM198-enriched Western diet develops more stable plaque phenotypes in tandem stenosis *Apoe*^{-/-} mice compared with a regular Western diet.

Although a very attractive target, MPO-Gd has been evaluated before in atherosclerotic mice¹⁰ and rabbits,¹¹ and inhibition of MPO has been investigated before.^{12,13} This study's innovative character

mainly revolves around the application of the relatively new tandem stenosis mouse model. Further investigations need to establish if this model truly exhibits characteristics of unstable human plaques, and whether extrapolation to a clinical setting is justified. As rightly pointed out by the authors, MPO therapy promotes plaque stability when started at the same time as the Western diet, but real clinical promise needs to be demonstrated in a setting of established disease. Furthermore, it remains to be investigated if AZM198 therapy really polarizes established unstable plaques to a stable phenotype. Additionally, a comparison with recognized anti-atherosclerosis treatments such as interleukin-1 β or statins could facilitate an improved assessment of the actual therapeutic potential.

The imaging and therapeutic approach suggested by Rashid *et al.* holds promise and warrants additional investigations into the pathways through which the therapeutic benefits were achieved. Importantly, the imaging read-out may assist translational therapeutic evaluation as it allows detection of fast and subtle changes over time and thus guide further development.

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