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EDITORIAL

Platelet-derived MMP-2 in the prevention of plaque formation: how many strokes is par?

Judith M.E.M. Cosemans *

Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

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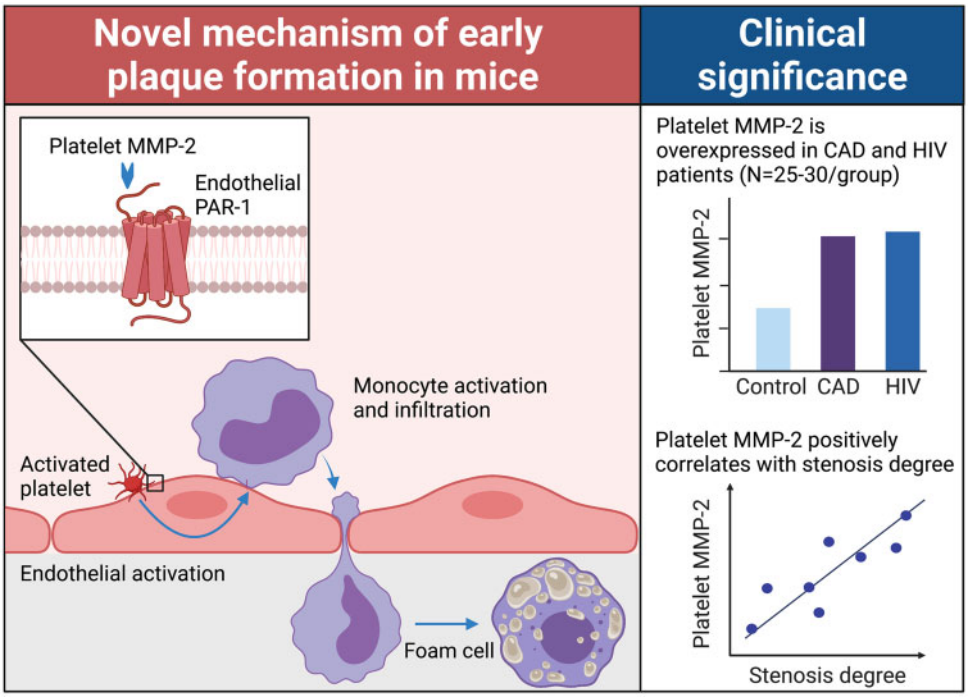
This editorial refers to 'Matrix metalloproteinase-2 on activated platelets triggers endothelial PAR-1 initiating atherosclerosis', by S. Momi *et al.*, https://doi.org/10.1093/eurheartj/ehab631.



Graphical Abstract Platelet-derived matrix metalloproteinase (MMP)-2 promotes early plaque formation in mice via activation of endothelial protease activated receptor (PAR)-1 and subsequent endothelial activation and monocyte intravasation. MMP-2 is overexpressed on the platelet surface in patients with coronary artery disease (CAD) and human immunodeficiency virus (HIV) compared with age- and sex-matched healthy controls. Platelet MMP-2 levels positively correlate with the degree of human carotid artery stenosis. This graphical abstract is based on the findings of Momi et al.[10] and was created with Biorender.com.

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.

* Corresponding author. Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Universiteitssingel 50, 6200 MD Maastricht, The Netherlands. Tel: +31 433881534, Fax: +31 433884159, Email: judith.cosemans@maastrichtuniversity.nl

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Atherosclerotic cardiovascular disease nowadays accounts for the majority of mortality worldwide. The adhesion of blood leucocytes to the inflamed arterial wall and their subsequent infiltration into the vessel wall is a key process in atherosclerotic plaque formation. Within the arterial wall, leucocytes are transformed into foam cells by uptake of lipids, leading to vessel wall thickening. Blood platelets are classically known for their vital role in bleeding and thrombosis, but also promote leucocyte infiltration via different mechanisms. Platelets can deposit chemokines on the inflamed endothelium and thereby recruit leucocytes, but can also directly activate leucocytes and endothelial cells. Platelets may also promote plaque formation by accelerating foam cell formation upon binding to oxidized LDL.[1] Although multiple receptor–ligand partners involved in platelet–endothelial and platelet–leucocyte interactions have been identified,[1] the full scope of interactions and mechanisms underlying the plaque-promoting effects of platelets and the translational relevance thereof for human atherosclerosis remain to be fully clarified.

Among the platelet-derived factors that might play a role in atherosclerosis are matrix metalloproteinases (MMPs). MMPs are a family of proteolytic enzymes that mediate physiological and pathophysiological extracellular matrix turnover. Platelets contain several MMP family members: MMP-1 (not in mice), MMP-2, MMP-3, MMP-12, and MMP-14 (reviewed by Gresele *et al.*).[2] Platelet thrombi have been shown to exert matrix-degrading activity *in vitro*[3] and *in vivo* locally at the site of plaque disruption,[4] which may facilitate leucocyte infiltration. As well as cleaving extracellular matrix components, MMP-1 and -2 can cleave protease-activated receptor (PAR)-1.[5,6] PAR-1 is a G protein-coupled receptor with important roles in haemostasis and inflammation.[7] Classically, PAR-1 is activated by thrombin through proteolysis and cleavage of the N-terminal extracellular domain of the receptor at the R41↓S42 site, leading to activation of Gq, Gi, and G12/13.[7] MMP-1 and -2 cleave PAR-1 at the LD39↓P40RS and TL38↓D39PR sites, respectively.[5,6] Each cleavage event results in an exposed amino acid sequence that acts as a 'tethered ligand' that reassociates with the main body of the receptor to trigger structural rearrangement and receptor activation. The generation of distinct tethered ligands by thrombin, MMP-1, and MMP-2 results in a diversity of signalling outcomes.[7] In platelets, PAR-1 activated by MMP-2 is found to trigger PAR-1 coupling to Gα12/13- and Gαq-coupled pathways, but not Gαi pathways.[5] Furthermore, integrin $\alpha_{IIb}\beta_3$ appears to be a necessary cofactor for platelet PAR-1 cleavage by platelet MMP-2.[5] Whether a cofactor is also required for endothelial PAR-1 cleavage by MMP-2 is unknown.

Recently, the Kuliopulos research group demonstrated a promoting role for MMP-1 in plaque formation in mice, mediated via interaction with endothelial PAR-1.[8] Here a pharmacological approach was employed, making use of the cell-penetrating PAR-1 pepducin PZ-128 and an MMP-1 inhibitor (FN-439, which is equally effective in inhibiting MMP-8 as well as MMP-1). The same group substantiated their findings using a genetic approach and demonstrated that $ApoE^{-/-}$ mice, deficient in the human MMP-1 orthologue, MMP1a, have a 50% reduction in plaque size when compared with $ApoE^{-/-}$ mice.[9] Interestingly, both MMP1a deficiency and chronic treatment of $ApoE^{-/-}$ mice with the PAR-1 inhibitor PZ-128 resulted in a significant 50% relative drop in circulating monocytes, indicating a potential systemic role for the MMP1–PAR1 system in regulating monocyte homeostasis.[9] Which cellular (platelets and/or monocytic) and/or plasmatic source of MMP-1 is/are responsible for the plaque-promoting role of MMP-1 is an open question.

In this issue of the *European Heart Journal*, Momi *et al.*[10] investigated the role of platelet MMP-2 in plaque formation in mice using a genetic approach. To enable hyperlipidaemic plaque formation, the LDL receptor (LDLR) gene was knocked out and mice were fed an atherogenic diet. The results indicate that LDLR/MMP-2 double knockout mice develop reduced intima media thickening of the femoral artery upon photochemical injury and reduced atherosclerotic plaques in the aorta when compared with $LDLR^{-/-}$ mice. Similar results were obtained with $LDLR^{-/-}$ mice transplanted with bone marrow from $MMP\text{-}2^{-/-}$ mice, suggesting that haematopoietic cells (leucocytes, platelets, and/or red blood cells) are responsible for the observed effect. Importantly, although MMP-2 deficiency impairs platelet adhesion to the exposed extracellular matrix at the arterial shear rate,[3,10] adhesion to activated endothelium under both the venous and arterial shear rate is unaffected,[10] suggesting that the observed reduction in plaque size is not caused by reduced platelet adhesion. Using a strategy of depleting mice for platelets and subsequently infusing isolated activated platelets from mice deficient in LDLR, MMP-2, and LDLR plus MMP-2, the authors conclude that platelet MMP-2 is responsible for the plaque-promoting role of MMP-2 in their mouse model. An involvement of leucocytes was considered unlikely, considering the high purity of the infused platelets (99.995%). Similar to Fletcher *et al.*,[9] a lower level of infiltrated monocytes/macrophages was observed in aortic plaques. Momi *et al.*[10] also found a shift in macrophage type from M1 macrophages in aortic plaques of $LDLR^{-/-}$ mice to M2 macrophages, associated with an anti-inflammatory activity, in $LDLR^{-/-}/MMP\text{-}2^{-/-}$ mice. It would be relevant to determine whether circulating monocytes levels are altered by targeting MMP-2, as shown for MMP-1,[9] which has apparently not been done in the present study.

The molecular target and underlying mechanism mediating the role of platelet MMP-2 in plaque formation was investigated *in vitro*. Previously, the authors had already shown that platelet MMP-2 can cleave and active platelet PAR-1,[5] and now they demonstrate that purified MMP-2 can cleave PAR-1 on human umbilical cord vein (HUVEC) cells.[10] Moreover, they show that activated platelets induced PAR-1-dependent endothelial phosphorylation of Akt. With regard to functional responses, activated platelets from $LDLR^{-/-}$ but not from $LDLR^{-/-}/MMP2^{-/-}$, $MMP2^{-/-}$, or chimeric $MMP2^{-/-}$ mice trigger the endothelial expression of adhesion molecules (VCAM-1) and leucocyte transendothelial migration. Taken together, platelets expressing MMP-2 activate endothelial PAR-1, triggering endothelial Akt signalling, the expression of endothelium adhesion molecules, and subsequent leucocyte transmigration (see *Graphical Abstract*).

The authors also provide translational relevance of their mechanistic findings by showing that MMP-2 is overexpressed on the surface of circulating platelets in coronary artery disease and chronic human immunodeficiency virus patients compared with age- and sex-matched healthy controls. Importantly, platelet surface expression of MMP-2 positively correlated with the degree of carotid artery stenosis.[10] Whether platelet MMP-2 expression correlates with (other) markers of platelet activation, such as P-selectin, was not measured. Previously the authors demonstrated that patients with acute coronary syndromes (ACS) have increased levels of plasmatic MMP-2 in blood obtained from the lesion-containing coronary artery when compared with peripheral blood, suggesting that platelets release

MMP-2 into the circulation.[11] Indeed, platelets can both release and express MMP-2 on their surface.[3] Another line of evidence shows that human atherosclerotic plaques contain high amounts of MMP-2[12,13] and that enhanced MMP-2 expression in atherosclerotic plaques has been associated with a higher rate of subsequent ischaemic cerebrovascular events.[12] As well as MMP-2, MMP-1 levels are also significantly elevated in ACS.[9,14] MMP-1 levels were found to correlate with the number of stenotic lesions[9] and to be an independent predictor of all-cause mortality in coronary artery disease patients.[14] Whether MMP-1 levels are correlated with cardiovascular events is an open question for future research.

Taken together, Momi *et al.*[10] demonstrate a hitherto unknown mechanism of how platelets promote early plaque formation in mice, i.e. via platelet MMP-2-mediated activation of endothelial PAR-1 and subsequent endothelial activation and leucocyte intravasation. Combined with the evidence from mouse studies that MMP-1 also promotes plaque formation via endothelial PAR-1[8,9] and clinical evidence that MMP-1 and -2 levels correlate with atherosclerotic plaque burden,[9,10], the MMP–PAR-1 axis has emerged as an interesting therapeutic target for inhibition of plaque development. With regard to direct inhibition of MMP-1 and MMP-2, a current challenge is to obtain inhibitors that selectively inhibit MMP-1 or -2 instead of multiple MMPs. Efforts are underway to develop MMP-2- specific nanobodies.[15] To inhibit PAR-1 activities, a wide range of pharmacological agents such as antibodies, peptides, small molecules, pepducins, and parmodulins exist.[7] Of these, pepducins and parmodulins interact with the intracellular domains of PAR-1, competing with the binding of G proteins to PAR-1. The pepducin PZ-128 is currently being tested as an antithrombotic agent in the acute setting in the TRIP-PCI study (Thrombin Receptor Inhibitory Pepducin-Percutaneous Coronary Intervention). In a mouse study, it was shown to impair plaque formation to a similar extent to MMP-1 inhibition.[9] Making use of the diversity in signalling downstream of PAR-1 upon its activation by MMP-1 and -2 vs. thrombin may provide a promising avenue to selectively target the proinflammatory activity of PAR-1. How many strokes for par are required in this regard remains to be elucidated.

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