Nonremodeling Properties of Matrix Metalloproteinases
The Platelet Connection

Paul Jurasz, Ada W.Y. Chung, Anna Radomski, Marek W. Radomski

Matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), play an important role as mediators of tissue remodeling under physiological and pathological conditions.1 Recently, a number of contributions have shown that in addition to chronic remodeling reactions, MMPs are involved in acute biological reactions associated with discrete cell signaling such as regulation of vascular reactivity,2-4 leukocyte activation,5 and platelet function.6-12

The study by Galt and colleagues, published in this issue of Circulation Research, shows that MMP-1, yet another member of the MMP family of enzymes, primes platelets for aggregation.13 They have demonstrated that the outside-in signals delivered by MMP-1 markedly increased the number of protein phosphorylated in platelets. In this respect, MMP-1 acts similar to platelet receptor agonists such as ADP, collagen, and thrombin, which are known to stimulate protein phosphorylation.14 In addition, this work provides further evidence that some biological functions of platelets are regulated by MMPs.

Blood Platelets
Platelets are small (∼2 μm in size) anucleate cell elements that are produced by fragmentation of large mother cell megakaryocytes. Although the process of platelet production (thrombopoiesis) is regulated by thrombopoietin, which controls megakaryocyte proliferation, maturation, and platelet generation, some stages of thrombopoiesis are dependent on the activity of MMP-9.15

Platelets are involved in physiological hemostasis and pathological thrombosis. After accidental or pathological injury, platelets adhere to the damaged portion of the vascular wall initiating an intricate set of reactions that lead via platelet aggregation to the formation of hemostatic plug or occlusive thrombus. Complex platelet metabolic pathways are involved in regulation of platelet function (Figure).

Matrix Metalloproteinases and Platelet Hemostasis
Although collagennolytic activity of platelets was discovered by Chesney et al,16 as early as 1974, the molecular identity of enzymes involved remained unknown until the late 1990s. To date, four MMPs including MMP-1, MMP-2, MMP-3, and MMP-9 have been identified in human platelets.7,8,13 In resting platelets, these enzymes, similar to many cell types, are stored in the latent form. In difference to leukocytes where pro-MMP-2 and pro-MMP-9 are stored in specialized gelatinase granules,17 in platelets, pro-MMP-2 is found in the cytoplasm without an apparent association with α or dense granules.7

Platelet adhesion triggered by von Willebrand factor12 and platelet aggregation by collagen, thrombin and human cancer cells all result in the liberation of both pro-MMP-1 and pro-MMP-2.6,7,10,13 The enzymes translocate to the platelet surface membrane where they appear to colocalize in areas of cell contact with β3 integrins.7,13 The translocation of pro-MMP-1 and pro-MMP-2 to the cell surface is likely to provide stimulus for enzyme activation. Interestingly, pro-MMP-2 may be activated by the classical membrane-type (MT)-MMP/TIMP-2–dependent pathway9 or by a mechanism that does not involve TIMP-2.18 A significant part of MMP-2 may be released or shed to the extracellular compartment.6,7,10 The processes of translocation and release are tightly linked to platelet activation and are downregulated by endogenous inhibitors of platelet aggregation, nitric oxide (NO), and prostacyclin I2 (PGI2).6,10

What is the biological significance of this trafficking of MMPs in platelets? The experiments using molecular and pharmacological inhibitors, as well as recombinant MMP-1 and MMP-2, have shown that these enzymes may prime platelets for adhesion and aggregation.6,11-13 Moreover, MMP-2 is one of the mediators of tumor cell–induced platelet aggregation, the phenomenon that plays a role in the hematogenous dissemination of cancer.10 In contrast, MMP-9, which is expressed in platelets in lower amounts than MMP-1 or MMP-2,13 appears to counteract the platelet-aggregatory effects of MMP-2 and inhibits aggregation,8 whereas MMP-3 is devoid of any effects on aggregation.13

The mechanisms of interactions of MMPs with platelets are still being elucidated. It has been demonstrated that only activated, but not latent, MMP-1 and MMP-2 stimulate platelet function suggesting that a limited proteolysis might underlie the platelet activator actions of these compounds.5,13 The actions of MMP-2 may involve modification of major platelet receptor glycoproteins GP Ib12 and GP IIb/IIIa11 or their ligands, whereas those of MMP-1 are based upon stimulation of tyrosine phosphorylation and clustering of β3 integrins to focal adhesion points.13 Whether MMP-1 and/or other MMPs modulate tyrosine phosphorylation via interactions with specific platelet receptors remains to be studied. Although the actions of MMPs on platelets may not require...
inhibit platelet activation.\textsuperscript{6,9,18} In addition to endogenous inhibitors, a number of structurally unrelated chemical inhibitors of MMPs reduce platelet adhesion and aggregation in vitro\textsuperscript{6,9,12–13} Unfortunately most, if not all, chemical inhibitors of MMPs, although specifically inhibit the MMP family of enzymes, show a low level of selectivity toward individual members of this family. This relative lack of selectivity might be one of the reasons behind the lack of effectiveness of chemical inhibitors in the treatment of cancer.\textsuperscript{27} It is also important to note that not all pathways of platelet activation are MMP-dependent. For example, platelet aggregation induced by PAR-4 agonist, AYPGFK, is MMP-independent.\textsuperscript{19} On the other hand, MMP inhibitors may decrease a part of platelet activation that is not sensitive to inhibition with aspirin or ADP antagonists\textsuperscript{6,10,12} and potentially stabilize the atherosclerotic plaque. Therefore, the concept of selective pharmacological inhibition of MMPs to reduce thrombotic complications of vascular disorders is worth considering.\textsuperscript{6} Interestingly, PGI\textsubscript{2} and NO both decrease activation-induced release of MMP-2.\textsuperscript{6,10} Moreover, m7E3 (ReoPro, abciximab), a GP IIb/IIIa receptor antagonist, reduced the release of MMPs and attenuated the vascular injury in rats.\textsuperscript{28} This ability to modulate MMP release from platelets and the vascular wall is likely to contribute to the pharmacological profile of PGI\textsubscript{2}, NO, and m7E3 as potent antiplatelet agents.

\section*{Conclusions}
Over the past few years, MMPs have emerged as a novel system that regulates platelet function. Understanding the biological effects of MMPs on the vascular hemostasis and thrombosis may have basic, clinical, and therapeutic significance.

\section*{Acknowledgments}
The authors’ work is supported by the Canadian Institutes of Health Research.

\section*{References}
9. Kapes I, Elalany I, Sraer J-D, Hatmi M, Nguyen G. Platelet release of trimolecular complex components MT1MMP/TIMP2/MMP2: The intermediate formation of cellular mediators such as thromboxane A\textsubscript{2} and ADP.\textsuperscript{6,10,12} MMP-2 crosstalks with other platelet agonists including proteinase-activated receptor (PAR) peptides.\textsuperscript{19}

Of four known endogenous inhibitors of MMPs,\textsuperscript{20} only TIMP-1 and TIMP-4 proteins appear to be expressed in measurable amounts in platelets, and TIMP-4 may be associated with MMP-2.\textsuperscript{18} However, it is likely that TIMP-2 that is expressed in high amounts in the vasculature\textsuperscript{21} also contributes to the regulation of MMP actions on platelets in vivo.

\section*{Pathological and Pharmacological Significance}
Platelet thrombosis may complicate the course of vascular disorders including atherosclerosis and diabetes. The structural disruption of the vascular wall, plaque rupture, and thrombosis are the pathological hallmarks of the coronary artery disease and myocardial infarction. MMPs are overexpressed at the site of human atheroma, and they may cause the matrix weakening and plaque rupture.\textsuperscript{22} Moreover, the release of MMP-2 exaggerates myocardial ischemia-reperfusion injury.\textsuperscript{23} Therefore, it is likely that the release of MMP-1, MMP-2, and MMP-9 from platelets, the vessel wall, myocardium, and monocytes\textsuperscript{9,12,13,24} may contribute to the thrombotic complications of atherosclerotic plaque rupture. The release of MMP-2 may also play a role in the thrombotic and inflammatory complications of the use of extracorporeal circulation\textsuperscript{25,26} and contribute to the hematogenous cancer metastasis.\textsuperscript{10}

Pharmacological studies have shown that human recombinant TIMP-2 and TIMP-4, but not TIMP-1, have the ability to


Nonremodeling Properties of Matrix Metalloproteinases: The Platelet Connection
Paul Jurasz, Ada W.Y. Chung, Anna Radomski and Marek W. Radomski

Circ Res. 2002;90:1041-1043
doi: 10.1161/01.RES.0000021398.28936.1D
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/90/10/1041

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/