Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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The vascular side of plasma kallikrein

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In this issue of *Blood*, Stavrou et al show that in plasma kallikrein–deficient mice ($Klkb1^{-/-}$), upregulation of the Mas receptor in the renin-angiotensin system leads to prostacyclin-mediated suppression of vessel wall tissue factor (TF), which leads to reduced thrombosis independent of reduced contact activation.[1]

Plasma prekallikrein is a liver-derived precursor of the trypsinlike serine protease plasma kallikrein (KK) and circulates in plasma bound to high-molecular-weight kininogen, the bradykinin (BK) precursor. The zymogen form is converted to KK by activated factor XII (FXII). KK proteolysis drives multiple cardiovascular reactions such as FXII zymogen activation in the intrinsic pathway of coagulation, BK formation via the kallikrein-kinin system, activation of plasminogen in fibrinolysis, formation of C3 and C5 in the classical complement pathway, and prorenin conversion in the renin-angiotensin system.[2]

KK-formed BK acts through stimulation of its G-protein–coupled B2 receptor to increase intracellular calcium that activates phospholipase A_2–forming arachidonic acid, leading to prostacyclin (PGI_2) formation in a cyclooxygenase-dependent manner. KK also activates prorenin to renin that generates angiotensin I from angiotensinogen. Angiotensin-converting enzyme (ACE) proteolyzes angiotensin I to angiotensin II and degrades intravascular BK. Thus KK regulates BK signaling on 2 independent levels: the protease forms BK by cleavage of kininogen and amplifies the BK-degrading renin-angiotensin system. Angiotensin-(1-7) [Ang-(1-7)] is the biological breakdown product of angiotensin II formed either by prolylcarboxypeptidase or the ACE homolog, ACE2, to activate the G-protein–coupled Mas receptor. Mas stimulation elevates nitric oxide and PGI_2, producing vasodilation and thrombosis protection and preventing cardiac hypertrophy and hypertension, respectively. Mas-deleted mice have increased vascular resistance, hypertension, and shorter times to thrombosis.[2,3]

KK also reciprocally activates zymogen FXII, and activated FXII initiates the intrinsic coagulation pathway via activation of factor XI (FXI). Both FXII- and FXI-deficient mice are protected from thrombosis in venous and arterial beds in multiple experimental models.[4] Defects in the FXII-driven pathway of coagulation are associated with delayed thrombosis in rabbits and baboon models.[5] Protection from thrombosis in FXII/FXI-deficient animals is caused by defective fibrin production,[4,6] and the fibrin-forming reactions are initiated by FXII-contact activators such as platelet polyphosphate, collagen exposed on ruptured plaques, or polymers that trigger FXII activation in clinically relevant scenarios, when blood becomes exposed to nonphysiologic surfaces during therapeutic procedures or extracorporeal circulation.[5,6] Reconstitution of FXII/FXI-deficient mice with human proteins fully restores FXII/FXI-stimulated clotting in plasma, fibrin formation, and thrombus formation in vivo.[4]

Prekallikrein-deficient ($Klkb1^{-/-}$) mice are protected from increased BK-driven vascular leakage in retinal vessels,[7] ferric chloride–triggered arterial thrombosis,[8] and hypotension in anaphylaxis models.[9] Protection conferred by KK deficiency in blood pressure regulation was similar to that observed in animals that are deficient in FXII or BK B2 receptors. Consistent with KK deficiency in patients (Fletcher trait) and deficiencies in FXII or FXI in mice and humans, $Klkb1^{-/-}$ mice have defective ex vivo plasma clotting that leads to a prolonged activated partial thromboplastin time (aPTT).

The study by Stavrou and coworkers shows that $Klkb1^{-/-}$ mice have defects in rose bengal– and ferric chloride–induced arterial thrombosis models. Reconstitution of $Klkb1^{-/-}$ mice with human KK to normal plasma levels corrected the prolonged aPTT but did not restore defective thrombus formation. In contrast to FXII/FXI-deficient mice, $Klkb1^{-/-}$ mice are not protected from collagen/epinephrine- and polyphosphate-driven lethal pulmonary embolism. In the collagen/epinephrine-induced pulmonary embolism model, $Klkb1^{-/-}$ mice have reduced lung perfusion from less BK formation, but they have vessel fibrin formation and platelet activation from acute lung injury like normal mice. Cumulatively, the findings indicate that chronic KK deficiency induces vascular alterations that cannot be reverted by short-term normalizing plasma KK levels and provides a thromboprotective activity in addition to reduced contact activation that is not observed in FXII/FXI-null mice.

In elegant comprehensive studies, Stavrou et al delineate a mechanism that explains the delayed thrombosis in KK deficiency unlike that observed in FXII/FXI-null states. KK deficiency suppresses BK- and BK B2-receptor expression. Compensatory mechanisms become upregulated to counterbalance reduced BK formation. In the absence of BK delivery to tissues, the receptor Mas becomes overexpressed in the presence of normal Ang-(1-7). Higher Mas with normal Ang-(1-7) levels results in increased PGI_2. PGl_2 upregulates expression of vascular sirtuin 1 (Sirt1) and Krüppel-like factor 4 transcription factors that culminate in suppression of vascular TF. This cross-system thrombosis-delay phenotype has been previously described by the same group in BK B2-receptor–deleted mice.[10] Taken together, these data link KK to vessel TF expression and show that the Mas receptor–PGl_2 pathway contributes to thromboprotection in KK (but not FXII) deficiency states. The thrombosis protection mechanism in *Klkb1*$^{-/-}$ mice is the opposite of the mechanism for increased thrombosis in the *Cox2*$^{-/-}$ mice that have reduced PGI_2, with vessel wall Sirt1 reduced and TF increased.

In sum, this study identifies an unexpected pathway whereby KK regulates TF expression that is mediated by the kallikrein-kinin and renin-angiotensin systems independently of FXII contact activation. The pathway provides an extra antithrombotic activity through elevated PGI_2. The novel pathway elaborated by these in vivo studies provides an attractive antithrombotic target. If murine findings have implications for human disease, long-term KK inhibition may result in higher levels of plasma PGI_2 and interfere with vessel-wall TF expression without platelet function inhibition and increased bleeding risk. Such therapy may be a potential adjunct to reduce arterial thrombosis risk, such as that seen in myocardial infarction and stroke.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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