Conflicting Forces of Warfarin and Matrix Gla Protein in the Artery Wall

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Fourteen years ago in this journal, Price and colleagues reported that 2 weeks of warfarin treatment in young rats "caused massive focal calcification of the artery media" (1). It had been known for years that warfarin could induce mineral deposition in the arteries of rodents (2), and the phenomenon was so robust that warfarin was often used as an experimental model for vascular calcification, but the mechanism was unknown.

In this issue, Tantisattamo and colleagues (3) provide evidence from a large sample of patients that the prevalence of vascular calcification detected by mammography is greater in women taking warfarin and that it appears to increase with duration of warfarin therapy. If one accepts the fair assumption that calcification of arteries in breast tissue reflects that occurring in other branches of the vascular tree - probably a fair extrapolation - this finding has enormous translational impact, given the morbidity and mortality associated with vascular calcification and given the millions of patients who use warfarin (4).

Their work ties together a series of prior laboratory observations. Twenty one years ago, calcified human carotid atherosclerotic plaque were found to express osteogenic differentiation factors, including bone morphogenetic protein (BMP)-2 (5) Four years later, skeletal biologists found, unexpectedly, that mice deficient in a cartilage protein, matrix Gla protein (MGP), develop widespread aortic and arterial calcification (6). How MGP could inhibit mineralization was uncertain, but such gamma-carboxyglutamic acid (Gla)-containing proteins are known for their ability to bind calcium and hydroxyapatite, so it was surmised that MGP inhibits mineralization by directly binding and limiting calcium mineral crystal growth, which it may in part. However, the late Marshall Urist, in his work leading to the discovery of BMP activity (before the protein had been purified), noted -- as a methodological aside -- that extraction of BMP activity from skeletal bone was extremely difficult unless MGP was removed first (7). Based on that clue, Bostrom tested and found that MGP binds BMP and inhibits its osteoinductive function (8). Importantly, Schurgers and colleagues (9) demonstrated that MGP function depends on its gamma-carboxyglutamatic acid residues. These residues arise via a post-translational modification that requires an enzyme that, in turn, requires vitamin K as a co-factor. Warfarin blocks this action of vitamin K on gamma-carboxylation in general, though its intended targets are Gla

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proteins other than MGP. Altogether, these observations indicate that warfarin blocks activation of matrix Gla protein, preventing both its opposition to BMP and preventing its direct opposition to mineral apposition. In effect, canceling out the double negatives, warfarin enables mineralization.

To project other possible consequences of MGP dysfunction in the context of warfarin, one may again consider the extreme case of the MGP knockout mouse. Unexpectedly, these mice develop widespread arteriovenous malformations (AVMs) in lungs, kidneys and brain, resulting in increased risk for intracranial bleeding and stroke (10). These knockout mice also have excessive angiogenic sprouting (11), which may result in a more friable vasculature. AVMs are also found in hereditary hemorrhagic telangiectasia (HHT) 1 and 2, which results from mutations in the endoglin (*Eng*) (HHT1) or activin receptor-like kinase 1 (*Alk1*) (HHT2) genes(12). Endoglin is a co-receptor required for ALK1 signaling. However, because the prevalence of AVMs in both mice and humans with HHT is significantly less than 100%, it has been suggested that a "secondary insult" is necessary to trigger AVMs in subjects with HHT2 (12). Since MGP is a downstream target of ALK1 in a feedback loop responsive to BMP activity (10), it is likely that alterations in ALK1 also alter the MGP response. In adult life, although the level of BMP activity may be so low as to have little or no consequence, a "secondary insult" may occur to induce BMP, such as inflammation, disturbed flow, or vascular trauma (13). In that case, without the customary MGP response as mediated by ALK1, there is unopposed action of BMP, which may trigger initiation of AVMs and pathological neoangiogenesis.

Altogether, these consideration raises a speculative concern that patients on warfarin may have increased propensity for developing AVMs or abnormal angiogenesis, perhaps more fragile microvasculature, and, thereby, an increased risk of bleeding. Such vascular abnormalities may contribute to the dramatic increase in risk of intracranial hemorrhage seen with higher doses of warfarin (14), which has been generally attributed to impaired coagulation. Such an effect of warfarin on the vasculature may even diminish the capacity of vitamin K as an antidote (15). Interestingly, in excess, MGP prevents the completion of development of the pulmonary arterial tree in mice (16) suggesting that MGP, though first recognized as a cartilage matrix protein, may serve as a key safeguard for the competence and solvency of the vasculature.

Finally, it is important to bear in mind the clinical benefits of warfarin in preventing thrombotic events, such as in patients with atrial fibrillation, prosthetic cardiac valves, and deep vein thrombosis. There are now alternative anticoagulants that act through mechanisms other than vitamin K antagonism. However, their effects on vascular calcification have not been established. Further clinical research -- similarly controlled for renal disease and atherosclerotic risk factors – is warranted to test for effects of warfarin as well as alternative anticoagulants, not only on coronary, carotid, and aortic calcification, but also on angiogenesis, arteriovenous malformations, and other vascular pathology.

Acknowledgments

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