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EDITORIAL COMMENT

PCSK9-Inhibition to Quench Vascular Injury in Chronic Limb Threatening Ischemia*

Ross Okazaki, MD, Naomi M. Hamburg, MD, MS

As the worldwide population ages, the burden of atherosclerotic disease including peripheral arterial disease (PAD) increases. Progression of PAD causes blood flow obstruction and hypoxia to the extremities. In the most severe form, critical limb ischemia or chronic limb-threatening ischemia (CLTI), PAD can result in limb loss through tissue ulceration and infection. Current medical therapy for CLTI mirrors that of PAD in general including risk factor control, but there remains a lack of proven medical therapies to reverse advanced ischemia. Recent randomized control trials for the management of CLTI have primarily addressed questions related to revascularization such as the benefit of early surgical vs endovascular intervention[1] and the limb-related benefits of low-dose rivaroxaban plus aspirin after revascularization.[2]

Over the past decade, there has been increasing interest in a powerful class of drugs that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 regulates the low-density lipoprotein receptor, and PCSK9 inhibitors cause dramatic reductions in low-density lipoprotein (LDL) cholesterol levels. When treated with PCSK9 inhibitors, patients with PAD experienced lower levels of major adverse cardiovascular events as well as reduced risk of major adverse limb events.[3] Whether PCSK9 inhibitors are beneficial or cost-effective in patients with preexisting CLTI has not yet been studied in large prospective trials.

Recent studies have sought to study PCSK9's role in atherogenesis; however, its role in PAD beyond its lipid-lowering effects have yet to be proven. The most well understood mechanism of PAD is the formation of LDL-rich atherosclerotic plaques that line the blood vessels. Obstructive plaques cause reduced distal blood flow, hypoxia, cell dysfunction, and death to distal tissue. Although hypoxia has long been known to induce apoptosis in cells, another pathway called pyroptosis has been more recently identified and shown to play a role in hypoxic cell death. Pyroptosis involves a series of inflammasome-mediated steps that result in cell swelling and loss of cellular membrane integrity that is mediated by the gasdermin family of proteins. In this issue of *JACC: Basic to Translational Science*, Zhang et al[4] sought to explore a novel role of PCSK9 in hypoxia-induced endothelial cell pyroptosis and its role in CLTI.

Their work started with a series of experiments using cultured endothelial cell exposure to hypoxia to model CLTI. An unbiased approach with RNA sequencing identified PCSK9 as a key up-regulated pathway. Genetic manipulation of PCSK9 levels altered levels of pyroptosis-mediated cell death in hypoxia conditions along with improvements in functional assays relevant to angiogenesis. Downstream mechanisms mediating the role of PCSK9 included modulation of mitochondrial function through translocation of Smac, a caspase activating protein, from the mitochondria to the cytoplasm. In a murine model of hindlimb ischemia, overexpression of PCSK9 through whole-body adeno-associated virus impaired the angiogenic response, whereas the

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From the Whitaker Cardiovascular Institute and Section of Vascular Biology, Boston University Chobanian and Avedisian School of Medicine, Boston, Massachusetts, USA.

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opposite was observed in the PCKSK whole-body knockout animals. The relevance in human CLTI was evaluated by demonstrating higher circulating levels of PCSK9 compared with non-PAD control subjects. Qualitative immunofluorescence microscopy of the amputated limb of a CLTI patient indicated higher PCSK9 expression in the endothelium in the distal (more ischemia) compared with the more proximal (less ischemic) tissue.

Overall, these findings indicate a role of PCSK9 and endothelial cell pyroptosis in response to hypoxia that may be favorably modulated by PCSK9 inhibition. Prior work has shown that PCSK9 inhibition improved arterial disease because of its lipid-lowering effect and its direct role in atherosclerosis via its interaction with the NLRP3 inflammasome. The present study shows that attenuation of PCSK9-mediated hypoxia-induced pyroptosis can occur on the cellular level independently of atherosclerosis and that these effects may underly another mechanism of the benefit of PCSK9 inhibitors. The work also highlights the significance of endothelial mitochondrial function in determining the response to ischemia. The functional impact of PCSK9 in the ischemia limb points to a novel mechanism for the clinical observation of reduced major adverse limb events with PCSK9 inhibitors. It is possible that direct endothelial protective effects lead to an improved angiogenic response that supplements the lipid-lowering effects.

It is important to note that several remaining questions and limitations exist. There is a general concern regarding the translational gap between the acute surgical hindlimb ischemia model performed in young animals and the chronic ischemia that afflicts older patients with CLTI and relevant comorbidities. The animal model used did isolate the impact of PCSK9 inhibitors from atherosclerosis because the animals used were not on a hyperlipidemic background. However, it remains possible that the whole-body approaches (both for the overexpression and knockout) may have had liver-directed and/or lipid effects of modulating PCSK9 that mediated the ischemic impact. It would be important to conduct future studies using an endothelial-specific approach to confirm the vascular benefits of PCSK9 inhibitors. In addition, the translational findings included are limited without information about the clinical status of the CLTI patients or the temporal collection of the plasma compared with any procedures or medical therapies. Additionally, given the patients with CLTI had higher LDL cholesterol levels, it may be that the higher circulating PCSK9 levels were driven by cholesterol and not by PAD or limb ischemia. The data from human ischemic limb in CLTI appears to be only from 1 amputated sample and needs confirmation in a larger number of individuals to relate directly to measures of ischemic burden.

Future directions include investigation into the mechanism by which hypoxia induces PCSK9 expression. Additionally, further investigation into the role of endothelial mitochondrial functioning would also be of interest. Although endothelial cells are not as energy hungry as, eg, skeletal muscle, the endothelium is sensitive to ischemic stress and mitochondria have been recognized to be responsible for several essential roles including reactive oxygen species production, programmed cell death regulation, angiogenesis promotion, and inflammatory activation.

The results of the present study would suggest that the cardiovascular benefits of PCSK9 inhibition in PAD extend beyond their positive effect on LDL cholesterol and atherosclerosis. Unfortunately, the uptake of intensive lipid-lowering including the use of PCSK9 inhibitors in patients with PAD remains suboptimal. Several barriers exist including limited awareness of PAD, the need for multidisciplinary teams to coordinate CLTI care, as well as lack of access to PCSK9 inhibitors because of coverage and price barriers in the United States. Health disparities also drive differences in uptake of medical therapies and who has access to newer treatment approaches for PAD.[5] Patients with CLTI are often at higher cardiovascular risk and derive the greatest benefit from intensive medical therapy approaches. The translational study by Zhang et al[4] supports the development of additional clinical evidence to evaluate the impact of PCSK9 inhibitor medications in patients with advanced PAD with a focus on prevention of adverse limb events. It remains critical to identify patients with PAD early and implement intensive risk factor control along with appropriate antithrombotic therapy to save both lives and limbs.

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ADDRESS FOR CORRESPONDENCE: Dr Naomi M. Hamburg, Whitaker Cardiovascular Institute, Boston University Chobanian and Avedisian School of Medicine, 72 East Concord Street, Boston, Massachusetts 02118, USA. E-mail: nhamburg@bu.edu.

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