Critical limb ischemia is associated with significant morbidity and mortality. Revascularization is not always feasible or successful. Consequently, in a worryingly high percentage of patients, amputation is the only option. It is hoped that a wider understanding of the molecular mechanisms underpinning limb ischemia can help develop new and truly revolutionary therapeutic strategies.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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S100A1: A Novel and Essential Molecular Component for Postischemic Angiogenesis

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In this issue of Circulation Research, Most et al propose the S100 protein A1 (S100A1) as a novel therapeutic target in limb ischemia. S100A1 is one of the several EF-hand type proteins, which is characterized by helix-loop-helix EF-hand calcium-binding domains and responds to variations in calcium levels with conformational changes regulating the interaction with protein targets. S100A1 is known to be expressed in striated muscles.

Here, Most et al show that S100A deficiency in vascular endothelial cells reduces their capacity to mount an appropriate angiogenic response. They have performed an in-depth mechanistic investigation after observing a dramatic reduction in S100A1 levels in muscular biopsies from critical limb ischemia patients compared with healthy controls. This is a successful example of reverse translational research.

The team already reported that S100A1 is expressed in endothelial cells, where it promotes calcium-dependent endothelial NO synthase (eNOS) activity. Using a global gene knockout murine model for S100A1, they demonstrated that S100A1 deficiency results in endothelial dysfunction and impaired vascular relaxation capacity. The new article goes further, investigating the expression regulation of S100A1 by hypoxia and the mechanisms linking S100A1 and eNOS function. The authors elegantly demonstrate that direct calcium-dependent interaction between S100A1 and eNOS promotes NO generation in endothelial cells. In addition, S100A1 attenuates protein kinase C activity, preventing inhibitory phosphorylation at the threonine-495 eNOS site (Figure). A deficiency in S100A1 seems to compromise fundamental functional characteristics of endothelial cells, including the ability to proliferate, migrate, and form capillary-like tube networks in vitro. Furthermore, the authors provide evidence of the importance of S100A1 in postischemic angiogenesis by inducing limb ischemia in S100A1 knockout mice and wild-type controls. Under ischemic conditions, S100A1 deficiency additionally affects vascular endothelial growth factor signaling by upregulating vascular endothelial growth factor-A expression (presumably via increased hypoxia-inducible factor 1) and hastening degradation of the vascular endothelial growth factor receptor 2 (possibly via increased protein kinase C activity), thus impairing the proangiogenic potential of vascular endothelial growth factor-A overall.

The authors show that hypoxia regulates S100A1 expression in cultured endothelial cells. Here, hypoxia has been modeled by reducing oxygen concentration to 2%. Interestingly, 24 and 48 hours of hypoxia dramatically reduce S100A1 levels in endothelial cells. However, a shorter exposure (2 hours) does not result in S100A1 loss, but rather it augments S100A1/eNOS binding. Hence, it would be important to understand the fine regulation exerted by different degrees of hypoxia and other ischemia-related environmental perturbations, which could affect S100A1 expression and function in endothelial cells. In addition, it would be tempting to speculate that ischemic preconditioning may protect endothelial cells from S100A1 loss. In this context, it should be considered that, unlike myocardial infarction, human limb ischemia is not a sudden event. Consequently, endothelial cells may have the opportunity to mount protective responses in this clinical setting.

As previously mentioned, this study finds S100A1 to be reduced in muscle biopsies from critical limb ischemia patients. To understand the endothelial component in S100A1 downregulation, the authors compare the in vitro SA100A1 expression changes in response to hypoxia between endothelial cells and skeletal myocytes. As a result of a smaller decrease in myocytes, they speculate that endothelial cells are principally responsible for the overall SA100A1 loss in critical limb ischemia biopsies. Nonetheless, a human critical limb ischemia–affected extremity is a complicated system, in terms of both cellular components and altered environmental conditions (hypoxia not being the only one). This research should be further enhanced by work on endothelial cells prepared from muscle biopsies.

As this study originates from work on clinical samples, it is tempting to speculate on the therapeutic potential of S100A1 and on the possible approaches to replenish the expression and activity of this protein in diseased blood vessels. To what extent S100A1 needs to be restored, and for how long, remains an open question. Considering that wild-type mice subjected to ischemia recover despite S100A1 downregulation, one can speculate that although complete abrogation of the protein is deleterious, even low levels may suffice...
to achieve functional neovascularization. An obvious option for stimulating S100A1-mediated angiogenesis is the use of gene therapy. Intravascular viral vector-mediated S100A1 gene delivery might benefit patients undergoing revascularization for critical limb ischemia. In this setting, endothelial cell targeting could be achieved because of improvements in adenovirus vectors. Adeno-associated vectors are also an attractive vehicle owing to prolonged transgene expression and the availability of a variety of serotypes, thereby reducing immunogenicity issues. The authors have developed exciting preclinical studies using adenov-associated viral vectors to increase S100A1 levels in the infarcted and failing heart. Nonetheless, similar to the native protein, transgenic wild-type S100A1 could be downregulated by ischemia. To address this issue, future studies that enable an understanding of the hypoxia-sensitive transcriptional and posttranscriptional mechanisms (possibly including microRNAs) regulating S100A1 expression should be developed to design hypoxia-resistant mutant forms of S100A1. An alternative to the single-shot gene delivery approach could be represented by frequent recombinant S100A1 protein injections directly into ischemic limb muscles. Importantly, extracellular human S100A1 protein can be internalized by cardiovascular cells and reduce apoptosis. It would be important to investigate the therapeutic potential of S100A1 gene and protein delivery in preclinical limb ischemia models (including in the S100A1 knockout mice). However, the authors have instead focused their in vivo experiments on the systemic use of an NO donor diethylenetriamine/NO (DETA/NO), which is not a specific approach in rectifying a deficiency in S100A1. As an alternative to DETA/NO, NO-releasing variants of drugs commonly used in vascular patients could be considered. Finally, protein kinase C inhibitors may rescue postischemic angiogenesis. A large number of limb ischemia patients suffer from diabetes mellitus. Diabetes mellitus impairs eNOS function and NO production. It would be interesting to understand whether the ischemia-induced decrease in endothelial S100A1 is more pronounced in diabetic subjects and contributes to diabetes mellitus–associated vasculopathies. Furthermore, future studies should investigate whether S100A1 is involved in coronary endothelial cell responses to ischemia. S100A1 gene therapy already proved useful for preserving postinfarct cardiac remodeling and function in animal models, which could be, in part, explained by improved reparative angiogenesis and blood flow recovery.

Finally, the authors have showed that bradykinin stimulates S100A1–eNOS interaction. We have previously described that the bradykinin-producing enzyme tissue kallikrein promotes therapeutic angiogenesis in limb ischemia models, with eNOS playing an essential role in this action. Hence, it is tempting to speculate that the proangiogenic action of the kallikrein-kinin system could be mediated by S100A1. Most et al do not investigate this hypothesis, which could be the objective of a future study.

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