Small Molecule Approaches for Promoting Ischemic Tissue Vascularization

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Angiogenesis and arteriogenesis identify 2 specific processes that lead to the formation of new capillaries and the development of collateral vessels from pre-existing arterioles, respectively. Both processes characterize tissue repair and remodeling occurring in acute and chronic ischemic vascular diseases, and both processes represent the final targets of therapeutic angiogenesis aimed at providing an alternative treatment strategy for patients with lower limb ischemia and coronary artery disease.

Angiogenesis and arteriogenesis are driven by distinct, but partially overlapping, cellular and molecular pathways. Whereas the role of hypoxia and angiogenic inducers seems to be the trigger for pathological angiogenesis (ie, tumors, inflammatory chronic diseases, retinal maculopathy), fluid shear stress might be the most important stimulus for initiation of arteriogenesis. Besides these specific initial triggers, angiogenesis and arteriogenesis share growth factors, chemokines, proteases, inflammatory cells, and bone marrow-derived cells, which play quantitatively different roles in sustaining and refining these mechanisms of vascularization.

A paradigmatic example of these differences is the differing roles played by inflammatory cells in angiogenesis and arteriogenesis. In fact, angiogenesis may also occur in the absence of leukocyte recruitment. On the contrary, fluid shear stress activates the expression of multiple genes in endothelial cells (EC) aimed mainly to trigger the attraction and adhesion of circulating blood cells, which are necessary and sufficient functions to maintain arteriogenesis.

In the last 10 years, relevant strides have been made with respect to therapeutic angiogenesis. Proof-of-concept evidence for therapeutic growth factor, both gene and protein-based approaches, has yielded variable results. This limits our ability to draw firm conclusions. A second and more recent therapeutic approach has been supported by in vitro experiments and preclinical models demonstrating that after an ischemic injury, bone marrow mobilizes cell precursors, most probably of myeloid origin, which sustain angiogenesis and arteriogenesis by releasing soluble molecules around the nascent vessels and, to a minimal extent, by an incorporation in the newly formed vasculature. Even if the molecular and cellular bases of these observations are largely enigmatic, promising but not definitive conclusions come from trials using bone marrow–derived and peripheral blood-derived stem/progenitor cells in treatment of patients with acute myocardial infarction.

In this issue of Circulation Research, Murphy et al demonstrate that sokotrasterol sulfate, isolated from the marine sponge Topsentia ophirhaphidites, and a synthetic steroid analogue, 2β,3α,6α-cholestansulfate, are powerful inducers of therapeutic angiogenesis in a murine hindlimb ischemia model. Murphy et al also show that sokotrasterol sulfate and 2β,3α,6α-cholestansulfate activity strictly depends on sulfate residues. They clearly show that these 2 steroids are angiogenic in chick chorion allantoid membrane assay, and that the steroids generate stable and functional vessels in the ischemic tissues that persist after therapy interruption. Sokotrasterol sulfate induces in vitro proliferation, survival, and directional migration of EC. The specific upregulation of αVβ3 integrin, VEGF-A, and cyclooxygenase (COX)-2 are other features of the effect of this steroid on EC. The necessary role of these molecules in mediating the proangiogenic effect of sokotrasterol sulfate have been inferred by the use of specific inhibitors, thus allowing an hypothesis on its mechanism of action (Figure).

Integrin αβ heterodimers are major extracellular matrix (ECM) receptors that exist in different functional configurations with regard to their affinity for ligands. Integrin signaling is bidirectional. “Inside-out” signals regulate integrin affinity for adhesive ligands, and ligand-dependent “outside-in” signals regulate cellular responses to adhesion. Integrins play key roles in embryonic and postnatal angiogenesis. Their crucial role is underlined by emerging data that, during vascular formation, the activity of endogenous proangiogenic factors is counterbalanced by the activity of endogenous antiangiogenic factors, such as secreted class 3 semaphorins, and that regulation of integrin adhesive functions represent a crucial shared target for both classes of factors. Historically, a large amount of data pointed to αVβ3 integrin, a receptor for fibronectin, fibrin, and vitronectin as one the major players in blood vessel formation. Indeed, blockade of αVβ3 integrin with antagonists disrupts tumor and experimental angiogenesis. ECM of ischemic injured tissues is particularly enriched in the αVβ3 integrin ligands. Therefore, when activated by...
Putative mechanism of action of sokotrasterol sulfate on EC. Sokotrasterol sulfate seems to be an indirect angiogenic inducer, which promotes vascularization by regulating transcription of some genes including vegf, cox-2, and integrin αvβ3. Integrin αvβ3 cooperates with a VEGF-A-dependent autocrine loop occurring in angiogenic EC in promoting EC survival, motility, and proliferation. COX-2 is involved in a positive regulation of production and activity of VEGF-A and of αvβ3-dependent cell adhesion and migration. COX-2 metabolites may induce a proinflammatory environment, which could favor vascular arteriolarization (for details see text). Dotted arrows respectively indicates hypothetical activities.

The proper ECM, αvβ3 integrin may exert a proangiogenic effect in at least 3 different ways: (1) by regulating adhesive and migratory processes; (2) by triggering antiapoptotic signals; and (3) by forming a supramolecular complex with VEGF receptor-2 that enhances its sensitivity to the proliferative and motility effect of VEGF-A. Notably, angiogenic EC produce growth factors, including VEGF-A. Therefore, the increase of αvβ3 integrin and VEGF-A by sokotrasterol sulfate may allow speculation that this steroid enhances the VEGF-A-mediated autocrine loop, which is further enhanced by αvβ3 integrin upregulation.

The role of COX-2 in vessel formation has been established by connecting its prostanooid metabolites with VEGF-A production and action. COX-2 null cells do not produce VEGF-A, and COX-2 inhibitors prevent VEGF-A–mediated signals. On the other hand, VEGF-A increases COX-2 transcription. A more stringent link between the Murphy et al article and COX-2 activity in vascularization comes from the observation that COX-2 inhibition suppressed αvβ3-dependent EC spreading and migration. The molecular targets of COX-2 metabolites are Rac and cdc42, 2 members of Rho family GTPases that regulate cytoskeleton dynamics. Furthermore, COX-2 metabolites, in particular prostanoids and chemokines, the upregulation of COX-2 could indicate that sokotrasterol sulfate treatment is connected with an inflammatory condition, which in principle may favor vessel arteriolarization.

The effect of steroid in inhibiting and promoting vascularization has been well known for a long time. Angiogenic activity has been reported for ligands of the nuclear hormone receptor superfamily, such as androgens and estrogens. Recently, the molecular mechanism underlying angiogenic growth factors regulation by estrogens in the female reproductive tract has been elucidated by the finding that a functional estrogen responsive element is present in the promoter region of vegf. Therefore, the elucidation of transcriptome induced by sokotrasterol sulfate in EC will be of invaluable help to understand how this steroid acts on vasculature. In particular, the study of Murphy et al does not discriminate whether sokotrasterol sulfate induces the transcription of vegf, which in turn regulates that of COX-2 and the integrin function, or it has multiple activities with genes involved in angiogenesis. Transcriptome analysis will also bring insights on the acute and chronic potential adverse effects of the therapeutic use of sokotrasterol sulfate.

The Murphy et al article gives a new and interesting perspective of the management of therapeutic angiogenesis by introducing the first example of the efficacy of a small and synthetic molecule in promoting revascularization in ischemic tissues. Target therapy based on small molecules seems to be a rational and positive evolution of studies founded on gene or protein delivery. Knowledge of small molecule chemical structure may facilitate pharmacokinetic parameters and cost reduction.

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Disclosures
None.

References


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