

EDITORIAL COMMENT

Sonodynamic Therapy of Atherosclerotic Plaques

Breaking the Cycle*

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Over the last decades, atherosclerosis has become 1 of the leading causes of mortality, accounting for 17.9 million deaths—or 31% of all deaths—worldwide. This incidence is expected to rise further, considering that atherosclerosis represents an accumulation of vascular damage increasing in an aging society. The current hypothesis on the development and progression of atherosclerosis was proposed more than 2 decades ago (1): prolonged accumulation of lipids and associated lipoproteins—specifically apolipoprotein B as a component of low-density lipoproteins (LDLs)—leads to the generation of complexes between LDLs and the subendothelial extracellular matrix (proteoglycans in particular), triggering an activation of both endothelial cells as well as vascular smooth muscle cells (vSMCs). These now-activated cell types recruit macrophages to the vascular wall via chemoattractants such as monocyte chemoattractant protein-1 (from vSMCs), as well as the expression of endothelial adhesion proteins (intercellular adhesion molecule 1, vascular cell adhesion molecule 1, E-selectin, and P-selectin). Furthermore, oxidized LDL particles represent a source of increased oxidative stress, causing vSMC

apoptosis and proliferation and the production of further extracellular matrix (ECM) proteins. The latter contribute to LDL-ECM complexes, thus perpetuating a process of increased atherosclerotic lesion formation. In later stages of this process, atherosclerotic plaques can form a necrotic core with a thin fibrous cap, which are prone to plaque rupture, a process that can result in the complete occlusion of the vessel.

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In this inflammatory environment, macrophages phagocytose LDL-ECM complexes, inducing their transition into foam cells, which constitute the necrotic core of a thin-cap fibroatheroma. Upregulation of the hypoxia-inducible factor-1 alpha (HIF-1 α) in those macrophages leads to the secretion of the potent angiogenic factor vascular endothelial growth factor (VEGF). This process is enhanced by the presence of oxidized LDL, which can induce HIF-1 α independently of hypoxia. VEGF-A, acting mainly through the endothelial VEGF receptor 2, is a powerful driver of endothelial proliferation and tip cell-mediated angiogenic sprouting along a VEGF-A gradient, leading to an increase in vascularization. This newly formed vasculature infiltrates the plaque from the adventitial side, remaining, however, largely dysfunctional due to a lack of stabilizing factors, such as angiopoietin-1 or platelet-derived growth factor B. Consequently, the plaque neovasculature lacks mural cell recruitment and displays higher vascular permeability than resting mature microvessels, because of poorly structured cell-cell junctions. In addition, the plaque neovasculature does not seem to be properly perfused, such that the hypoxia in the necrotic core is not affected by the angiogenic sprouting of vessels into the plaque. Unfortunately, the immature vascular plexus

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does facilitate the recruitment of additional macrophages to the necrotic core, aggravating the cycle of inflammation present in atherosclerotic lesions (2).

Consequently, targeting the neovascularization of the atherosclerotic plaque has been intensively studied as a treatment option in atherosclerosis. Here, a disruption of VEGF-A-mediated activation of endothelial cells, which is produced in macrophages, appears promising. However, systemic therapies with anti-VEGF-A agents, such as the monoclonal antibody bevacizumab, do exhibit high rates of side effects (bleeding, hypertension), rendering their systemic application outside of cancer patients unlikely. To circumvent this problem, sonodynamic therapy (SDT) offers the possibility of reducing the macrophage number in atherosclerotic lesions, thus reducing the amount of VEGF-A present in the plaque, and reducing the stimulus for angiogenic sprouting of dysfunctional vessels. SDT utilizes compounds that accumulate in a tissue of choice and exert a cytotoxic effect after exposure to ultrasound (3). In the case of atherosclerosis, protoporphyrin IX and its precursor 5-aminolevulinic acid (5-ALA) have been shown to accumulate in macrophages in addition to malignant cells, for which their use was initially intended. Protoporphyrin IX generates toxic reactive oxygen species once activated via ultrasound through a still poorly understood mechanism and has been used in preclinical animal models of atherosclerosis.

In this issue of *JACC: Basic to Translational Science*, Yao et al. (4) demonstrate the efficacy of a novel compound—sinoporphyrin sodium (DVDMS)—as a sonosensitizer to inhibit the neovascularization of atherosclerotic plaques. Sinoporphyrin, a derivative of the photosensitizer Photofrin, has been demonstrated to possess an increased cytotoxic effect when compared with other commonly used sonosensitizers (protoporphyrin IX, hematoporphyrin, and Photofrin II). To investigate the efficacy of this novel compound, 2 animal models of atherosclerosis were utilized, namely a model of rabbit advanced femoral plaque formation (after injection of Russell's viper venom and histamine) and the classical model of atherosclerotic plaque formation in apolipoprotein E-deficient mice. In their *in vivo* studies, the authors demonstrate an increased macrophage apoptosis and a reduction in the density of the vasa vasorum after treatment with DVDMS-SDT accompanied by a reduction of overall plaque size. In subsequent *in vitro* experiments, Yao et al. (4) show an increased endothelial cell proliferation, reduced apoptosis, and

enhanced tube formation in human umbilical vein endothelial cells when co-cultured with THP-1-derived foam cells indicative of an angiogenic endothelial phenotype, which was abolished in DVDMS-SDT-treated cells without a direct effect of DVDMS-SDT on endothelial cells alone. Furthermore, the addition of VEGF-A to this co-culture setup was sufficient in restoring the proangiogenic phenotype in DVDMS-SDT-treated cells, highlighting VEGF-A as a potential effector growth factor in this interplay. Apart from the direct effect on the sprouting propensity of endothelial cells, the authors further demonstrate a decrease in the production of HIF-1 α , matrix metalloproteinase-2, and metalloproteinase-9, potentially resulting from an increase in macrophage caspase 3 activity after DVDMS-SDT elicited by the accumulation of reactive oxygen species in macrophage mitochondria.

Although the impact of DVDMS as a sonosensitizer on atherosclerotic plaque resident foam cells has been explored extensively in this paper, the assumed superiority of sinoporphyrin as compared with other potential sonosensitizers such as 5-ALA remains debatable. In previous works by the group, 5-ALA likewise was demonstrated to be capable of reducing atherosclerotic plaque formation, as well as macrophage density when used as a sonosensitizer during SDT (5). Furthermore, the question about the structure and functionality of the remaining vasa vasorum after the clearance of macrophages from the atherosclerotic plaque and thus removal of the constant angiogenic stimulus via VEGF-A remains. Although vessel density is reduced after DVDMS-SDT treatment, pericyte coverage and barrier capability—in short—the degree of functionality of the remaining vessels, remains unclear. Further insights need to be gained concerning these questions in the future, as an increase in functional vessels might improve the oxygen supply in the necrotic core, thus reducing the degree of oxidative stress, which could potentially breach the vicious cycle of continuous macrophage recruitment.

Intriguingly, the authors include a first clinical application of DVDMS-SDT in atherosclerotic patients in their study, in which they are able to demonstrate a reduction in plaque vessel density and a reduction in vessel inflammation measured via positron emission tomography computed tomography, mirroring the results obtained in the previous animal experiments. Although this represents an enticing outlook for a future clinical application, the question of the ideal sonosensitizer remains, particularly considering the unfavorable side effect profile of other

sonosensitizers. The authors furthermore recognize the need for proper randomized clinical trials to facilitate the transition of sonodynamic therapies from translational research to an established adjunctive therapy in vascular occlusive disease.

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