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Developments in the Role of Endothelin-1 in Atherosclerosis: A Potential Therapeutic Target?

Greg Sutton, Dan Pugh, and Neeraj Dhaun^o

Atherosclerosis is a progressive inflammatory disease of the arterial intima defined by fatty plaques within the vessel wall.¹ Plaques are characterized by the subendothelial build-up of low-density lipoprotein (LDL) leading to local inflammation and the accumulation of macrophage-derived foam cells. The development and subsequent rupture of atherosclerotic plaque leads to potentially catastrophic vascular events such as myocardial infarction and stroke. Thus, prevention of this largely asymptomatic condition is vital. Our understanding of the pathogenesis of atherosclerosis has progressed significantly over the last 3 decades, broadening the scope of preclinical studies and producing several exciting potential therapeutic targets. The role of the innate immune system and how it might interact with other key factors involved in atherogenesis have been the subject of much recent interest.^{2,3}

Endothelin-1 (ET-1) may represent a novel therapeutic target for the treatment and prevention of atherosclerosis particularly through its interactions with macrophages.⁴ ET-1 is the most potent endogenous vasoconstrictor produced primarily by the vascular endothelium. It has been implicated in endothelial dysfunction, inflammation, and vascular remodeling.^{5,6} It acts through 2 G protein-coupled receptors, the endothelin A (ET_A) receptor and the endothelin B (ET_B) receptor. Vascular smooth muscle cell ET_A receptors mediate vasoconstriction whereas ET_B receptors, mostly located on endothelial cells, mediate vasodilation. It is the actions of ET-1 through the ET_A receptor that are considered most important in cardiovascular diseases, such as hypertension, chronic kidney disease, and atherosclerosis.⁶

Circulating ET-1 is increased in patients with atherosclerosis.⁷ In 1995, Kowala et al. demonstrated that ET_A receptor antagonism reduced fatty streak development in hyperlipidemic hamsters.⁸ These studies and others proposed the rationale that ET-1 may have a role in the early inflammatory

phase of atherogenesis and that this may be amenable to treatment. Despite these findings, few clinical trials have attempted to address this. One study randomized 72 patients with non-obstructive coronary artery disease to receive either the selective ET_A receptor antagonist, atrasentan, or placebo for 6 months.⁹ The authors observed no significant difference in the progression of angiographic coronary atheroma, perhaps unsurprising given the relatively short time period of the study. Nevertheless, there was a significant reduction in blood pressure in the treatment group and work from the same group later demonstrated that 6 months of atrasentan improved coronary endothelial function in a similar group of patients. Thus, one might anticipate less atherosclerosis progression if these effects are maintained longer term.

In this issue of the *American Journal of Hypertension*, Zhang et al. explore the interplay between ET-1, endothelial cells, and macrophages within the context of atherosclerosis.¹⁰ They initially make 2 observations. First, that apolipoprotein-E knockout (ApoE^{-/-}) mice fed a high-fat diet have an increase in endothelial cell ET-1 protein expression within atherosclerotic plaques and second, that human umbilical vein endothelial cells (HUVECs) demonstrate an increase in ET-1 production on exposure to oxidized LDL (oxLDL). On the basis of these, the authors hypothesize that oxLDL stimulates endothelial ET-1 production, which then promotes progression of atheromatous plaque.

During a series of *in vitro* studies, using HUVECs adenovirally transfected to overexpress ET-1 (*et*HUVECs), Zhang et al. show that a combination of ET-1 overexpression and oxLDL promotes endothelial cell production of a range of adhesion molecules and chemokines. These effects are not seen with excess ET-1 alone and are greater than those seen with oxLDL. Monocyte chemoattractant protein-1 (MCP-1 or CCL2) and its receptor, CCR2, are among the chemokines

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assessed. These are key players in regulating the migration and infiltration of monocytes and macrophages.¹¹ Thus, this finding provides a natural progression to performing a co-culture of (both mouse and human) macrophages with *et*HUVECs. Here, the authors find that, whereas ET-1 alone has no effect, a combination of ET-1 and oxLDL promotes macrophage migration. Interestingly, when macrophages are cultured with conditioned medium from *et*HUVECs treated with oxLDL, they develop a more inflammatory/M1 phenotype and less of an anti-inflammatory/M2 one as demonstrated by upregulation of message encoding interleukin-6 (IL6), tumor necrosis factor- α (TNF α), and inducible nitric oxide synthase (iNOS) and downregulation of IL10 and mannose receptor. These findings are in line with previous work.¹² Further experiments demonstrate that all of the inflammatory effects of ET-1 and oxLDL on endothelial cells are dependent on an unblocked ET_A receptor and functional protein kinase C (PKC) signaling (Figure 1). PKC has been shown to regulate ET-1 expression in diabetes^{13,14} and its inhibition is being explored in a range of diseases.¹⁵

The authors then translate their *in vitro* findings *in vivo* using an ApoE^{-/-} mouse with vascular endothelial overexpression of ET-1 (eET-1/ApoE^{-/-}). They find that atherosclerotic plaque size is increased in these mice compared with relevant controls. In addition, plaques contained more cells expressing CD68, a protein highly expressed by cells of the monocyte lineage. Aortic tissue from these animals also showed heightened expression of pro-inflammatory but downregulation of anti-inflammatory cytokines. The authors did not treat these mice with ET receptor blockers, which might have further dissected the role of ET_A and/or ET_B signaling in the effects seen. An upregulated ET system has been linked to inflammatory cell migration into vascular tissue and reactive oxygen species production and so some assessment of vascular

function, e.g., using wire myography, might have been informative.¹⁶ Other work has shown that chronically elevated endothelial ET-1 leads to sustained blood pressure elevation and vascular and renal injury and that this is mediated *via* ET_A receptors.¹⁷

Recent work has shown that ET-1 is a potent chemoattractant for both mouse and human macrophages and that this is dependent on an unblocked macrophage ET_B receptor with some contribution of ET_A. This same study showed that ET-1 alone or in combination with either classical or alternative activation methods was unable to alter macrophage phenotype to any measurable extent.⁴ Zhang et al's finding that ET-1 alone does not influence macrophage phenotype is in keeping with these data. However, in their hands, ET-1 alone is insufficient to influence macrophage chemokinesis (unless in the presence of oxLDL), which is out of keeping with this earlier study. This may be due to the different experimental conditions used (e.g., endothelial cell line-derived ET-1 here vs exogenous ET-1).⁴

It is intriguing that ET_A receptor antagonism prevented the inflammatory effects of combined ET-1 and oxLDL on endothelial cells. Healthy vascular endothelial cells are considered to only express ET_B receptors,¹⁸ although there have been reports of endothelial ET_A expression in some disease states and specific cell lines.¹⁹ The authors used an ET_A receptor antagonist (BMS-182874) that is 1,000-fold selective for ET_A over ET_B so the effects seen are unlikely to be due to any functional ET_B blockade. Also, it is interesting that at baseline they found similar ET_A and ET_B expression in control HUVECs with *et*HUVECs expressing greater ET_A than ET_B. Thus, the findings from this study must be translated with some caution as they may be restricted to the cell lines used.

Both selective ET_A and mixed ET_{A/B} receptor antagonists (ERAs) are currently licensed and available in the clinic.⁶ Thus, the current studies would have benefited from additional investigation of combined ET_{A/B} receptor antagonism.

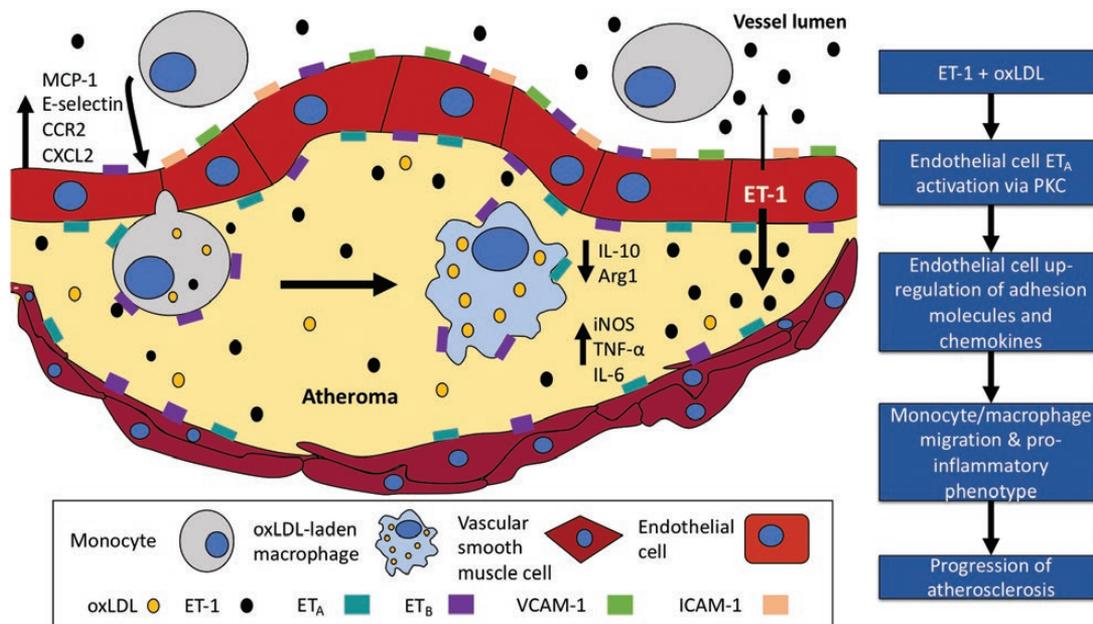


Figure 1. Potential role of endothelin-1 (ET-1) and oxidized LDL (oxLDL) on the development and progression of atherosclerosis.

Therapeutic blockade of the ET system has been investigated for several cardiovascular disorders. These agents effectively reduce blood pressure in treatment-resistant hypertension and reduce proteinuria on top of standard care in patients with chronic kidney disease (CKD).⁴ Indeed, the recently published study of diabetic nephropathy with atrasentan (SONAR) demonstrated a significant reduction in CKD progression with atrasentan compared with placebo in patients with diabetic nephropathy.²⁰ In addition, ERAs are first-line therapy for the management of pulmonary arterial hypertension, a rare but devastating condition. All these conditions are associated with accelerated atherosclerosis and so the authors should be commended for these data, which expand our knowledge with respect to the interaction between ET and innate immune systems in the development of atherosclerosis.

DISCLOSURE

None of the authors have any conflicts of interest.

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