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Linking regulation of nitric oxide to endothelin-1: The Yin and Yang of vascular tone in the atherosclerotic plaque

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A healthy endothelium prevents the development of atherosclerosis through protective effects on vasomotion, platelet adhesion, leukocyte trafficking, anti-inflammatory and anti-oxidant properties [1]. Nitric oxide and endothelin-1, autocrine and paracrine factors produced by endothelial cells, have opposing effects on smooth muscle cells contraction. The net balance between these pleiotropic molecules contributes to the regulation of local vascular tone. Nitric oxide (NO), the most potent vasodilatory molecule produced in the arterial wall, mediates endothelium-dependent relaxation (EDR). NO arises from the conversion of L-arginine to L-citrulline by the enzymatic action of an NADPH-dependent NO synthase (NOS) [2]. The endothelium produces NO by constitutive expression of the endothelial isoform of NOS (NOS3), which is activated by shear stress [3]. NO has a variety of functions, but its action as the predominant endothelium-derived relaxing factor (EDRF) is the most important for the maintenance of vascular homeostasis. Endothelin-1 (ET-1), which is encoded by the preproendothelin-1 gene (EDN1), functions as an opposing force on

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vascular tone, mediating vasoconstriction of vascular smooth muscle cells through binding to endothelin ET_A receptors [4]. ET-1 links causally to coronary artery disease. ET_A receptor (EDNRA) blockade inhibits whereas endothelium-restricted overexpression of EDN1 increases experimental atherosclerosis in mice [5,6], and non-coding variants that regulate EDN1 and EDNRA expression associate with human disease in genome-wide associate studies [7,8].

Given the opposite but complementary roles of NO and ET-1 it is not surprising that they are co-regulated by the same factors. In endothelial cell dysfunction and later stage atherosclerosis eNOS expression increases and the enzyme becomes uncoupled, generating the highly oxidant species superoxide instead of NO [9,10]. Risk factors for atherosclerosis such as dyslipidemia, diabetes, hypertension and smoking all reduce NO expression in cultured endothelial cells and impair EDR [11,12]. The opposite holds true for ET-1 function and expression, which increases in endothelial cell dysfunction and atherosclerosis [4,13]. There is some evidence that NO and ET-1 directly regulate each other to achieve vascular tone homeostasis. Stimulating the production of NO in endothelial cells can reduce ET-1 expression and production [14]. Similarly, ET-1 can directly induce the uncoupling of eNOS [10] whereas blocking ET_A receptors restores NO-dependent vascular function in mice with atherosclerosis [6]. These multiple mechanisms of counter-regulation between NO and ET-1 demonstrate the close control of vascular tone in health and disease. Identifying more molecular pathways that affect this tight balance is important for identifying therapies that affect the arterial wall.

In the current issue of *Atherosclerosis*, Rafnsson et al. [15] identify the direct effect of ET-1 on arginase expression and activity as a new mechanism that links ET-1 to NO function and EDR. With samples from the large human Biobank of Karolinska Endarterectomies (BiKE) the authors show that ET-1 and arginase pathway genes demonstrate similar patterns of expression. In RNA extracted from 177 carotid plaques there was higher arginase 2 (ARG2) and EDNRA gene expression compared with non-atherosclerotic iliac artery controls. Comparing symptomatic patients (defined as those who have experienced transient ischemic attack, minor stroke, or amaurosis fugax) to asymptomatic patients in their registry demonstrated higher EDN1 and EDNRB (ET_B receptor) expression in the plaques as well as augmented mRNA expression of ARG1, ARG2, EDNRA and EDNRB in the PBMCs. Immunohistochemical studies showed co-localization of arginase-1 and arginase-2 and ET-1 in the necrotic core of the plaque. The proteins seemed to also co-localize with macrophage marker CD68, suggesting the participation of plaque macrophages in the regulation of arterial tone in regions with atheromatous lesions. To validate the functional importance of these findings, the authors show that ET-1 stimulates ARG2 expression and activity (Fig. 1) in cultured human carotid artery endothelial cells and the THP-1 human macrophage cell line. The change in expression of arginase-2, but not arginase-1 in response to ET-1 is greater in ECs than in macrophages. Only in macrophages, however, did ET-1 stimulate the production of superoxide, as measured by ESR using 1-hydroxy-3-methoxycarbonyl* −2,2,5,5-tetramethylpyrrolidine as a spin trap, an effect that was abrogated by an arginase inhibitor.

The findings by Rafnsson et al. [15] support the opposite but complementary actions of ET-1 and NO in the development and progression of atherosclerosis. The abundant expression of ET-1 and its two receptors, ET_A and ET_B in late stage carotid plaques confirms the role of this potent vasoconstrictive pathway, as previously demonstrated in cultured ECs and atherosclerotic arteries [16]. One novel function of ET-1 appears to be upregulation of arginase-2 expression and function in cells found in the atherosclerotic plaque. Arginase is known to be a critical reciprocal regulator of NO production by competing with eNOS for the substrate L-arginine in endothelial cells (Fig. 1). By co-localizing ET-1 and arginase-2 expression to the necrotic core of the plaque and functional experiments in ECs and THP-1 macrophage the authors link ET-1 to decoupling of eNOS from production of NO to superoxide production via the depletion of arginase in both cell types [15]. The finding that ET-1 regulates arginase-2 expression and activity as well as arginase-2-derived superoxide production thereby inhibiting NO bioavailabilty is unexpected and novel. This observation adds to the understanding of the role of ET-1 in atherosclerosis and, possibly, other vascular diseases. Importantly, the effects seen in the cell studies were distinct, seen in either endothelial cells or macrophages. If relevant for human atherosclerosis, arginase-2-dependent effects of ET-1 could contribute to the anatomic and functional heterogeneity of atherosclerotic lesions [4,13]. Local arterial spasm favored by imbalance in the net actions of NO and ET-1 could explain why lesions of the same size and degree of luminal encroachment vary widely in their clinical expression and the temporal dispersion of events.

This study also has some limitations. Vascular smooth muscle cells determine atherosclerotic plaque progression but were not studied for their regulation of the ET-1/arginase pathway. Prior studies on arginase in VSMCs suggest they play an important role in mediating similar effects attributed to ECs and macrophages by Rafnsson et al. [15]. The aorta of rabbits with atherosclerosis exhibit increased expression of arginase-1 and arginase-2 [17]. Arginase promotes proliferation of vascular smooth muscle cells and is stimulated by oxidized LDL in both endothelial and intimal smooth muscle cells [18]. Moreover, angiotensin II, which accelerates atherosclerosis progression, stimulates vascular smooth muscle cell proliferation and fibrosis through arginase-1, but not arginase-2 [19]. These findings suggest that the mechanism for the beneficial effects of ET_A receptor-selective endothelin receptor antagonists (ERAs) in mice [6] and patients [20] with atherosclerosis may results in part from reduction in arginase expression in multiple vascular cells.

Future work to understand the exact cells which exhibit dysregulated ET-1, arginase-1, arginase-2 and NO function will be important for understanding how homeostasis goes awry in atherosclerotic arteries. Rafnsson et al. find that ET-1 expression is relevant in at least endothelial cells and macrophages, two cell types involved in the formation of fatty streaks as early as in utero [21]. Determining if certain subtypes of these cells, such as foam cells or VSMC-derived macrophages, display different patterns of gene regulation will be important. Indeed, expression of the arginase isoforms differs in macrophages with different functional polarization (“M1 vs. M2”) [22]. Advancements in droplet-based single cell RNA-sequencing now permit even finer analyses of cellular heterogeneity in human vascular disease [23]. Two recent papers have identified signatures of immune cells in human plaques [24] and VSMCs in mice with hypercholesterolemia due to loss of apolipoprotein E [25]. In

both cases distinct subsets of cells displayed characteristics implicated in plaque progression. These single cell transcriptional profiling methods allow for unbiased identification of all pathways upregulated in atherosclerosis. Rafnsson et al. [15] focus on the ET-1/NO axis, but unbiased analyses for mechanisms upregulated at different stages of disease will prove informative for uncovering new biology. Large repositories of human vascular tissue like the BiKE registry are an excellent resource for studies to profile the cells and biological pathways responsible for disease using new sequencing and bioinformatic technology.

Finally, the question as to whether and how endothelin receptor antagonists (ERAs), which limit atherosclerosis progression in mice and patients [6,20] might affect arginases deserves further study. It would not surprise if ERAs mediate some of their protective effects on atherosclerosis via inhibition of arginase. Moreover, it is possible if not likely that the improvement of NO-dependent vasodilatation seen after selective ET_A receptor blockade in atherosclerosis [6] involves opposing effects on both NO synthase and arginases which both share the same substrate: L-arginine (Fig. 1). The links between the ET-1/arginase/NO axis and expression of disease-causing genes can be unexpectedly complicated, but remain integral for the development of new therapies for vascular disease.

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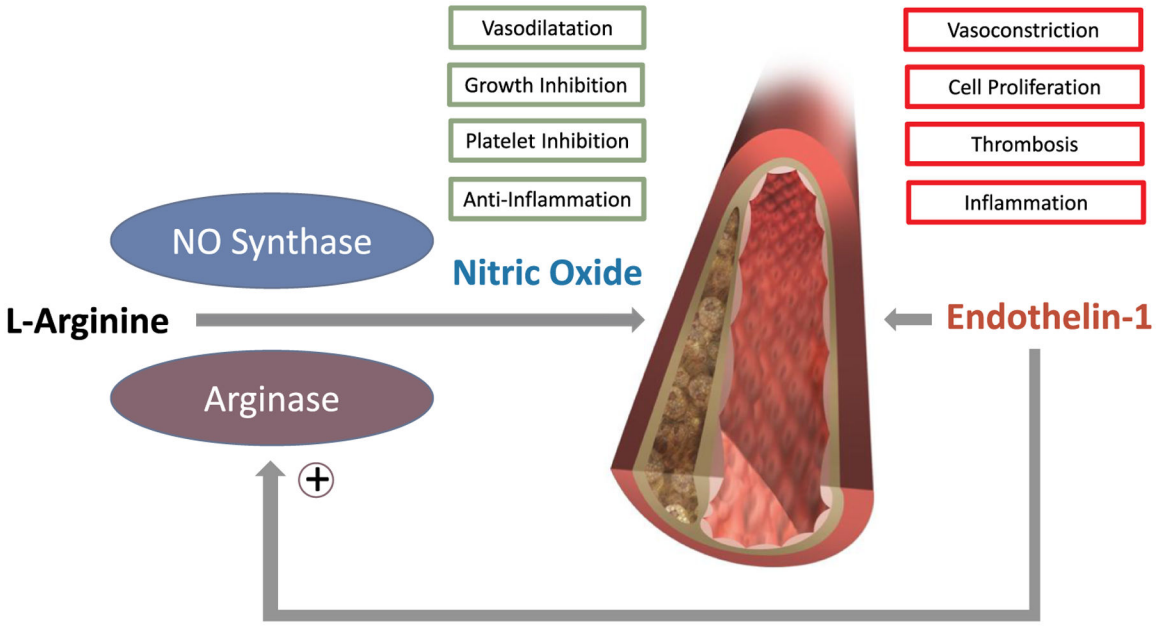

Fig. 1.

The opposite effects of NO and ET-1 on vascular function are counterbalanced in healthy tissue and dysregulated in atherosclerosis.

A new mechanism of regulation is the ability of ET-1 in vascular endothelial cells and macrophages to induce arginase expression, which then competes for L-arginine substrate with NO synthase, thereby reducing NO bioavailability.