Flow: The Signal of Life

Thomas F. Lüscher, Roberto Corti

Ever since the German physiologist Schretzenmayr at the beginning of the last century observed a widening of large arteries on exposure to increased flow, it was clear that blood vessels were not tubes, but able to react to forces exerted by the circulating blood. The sensor of flow was only discovered much later, when endothelial cells were recognized as a rich source of vasoactive mediators. At first the release of prostacyclin from cultured endothelial cells was shown to be shear stress dependent. The discovery of endothelium-dependent relaxations to acetylcholine in 1980 by Furchgott inspired a search for physiological stimuli for the putative endothelium-derived relaxing factor (EDRF). Indeed, in perfused femoral arteries of the dog, increases in flow markedly augmented the release of EDRF, a phenomenon that was soon confirmed in vivo as well.

The identification of EDRF as nitric oxide (NO) in 1987 and later the cloning of all three isoforms of the enzyme nitric oxide synthase (NOS) provided better tools to characterize the mediators of flow-dependent vasodilation. Inhibitors of NO such as N\(^{\text{G}}\)-monomethyl-l-arginine (l-NMMA) and N\(^{\text{G}}\)-nitro-l-arginine methyl ester (l-NAME) were able to prevent flow-mediated vasodilation in isolated arteries in animal models and in the human forearm. Thus, it became quite clear that shear stress exerted by the circulating blood elicits the release of NO from endothelial cells. Based on these findings, it was hypothesized that such a mechanism not only would provide higher vascular conductance (for instance in the coronary circulation under conditions of increased demand such as exercise), but also would ensure continued inactivation of circulating platelets in spite of the increased shear exerted on them. Furthermore, other protective effects of NO against the expression of inflammatory signals and vascular proliferation were thought to be of importance and used to explain the beneficial effects of exercise in the prevention of cardiovascular disease. Indeed, several studies documented the fact that prolonged exposure to shear stress not only increases the release of NO, but also the expression of the endothelial NOS (eNOS) enzyme. Furthermore, it was shown also in humans that exercise improves endothelial function in the forearm, as well as in the coronary circulation. Moreover, in patients with angina resulting from coronary disease a comprehensive exercise training proved to be as effective as coronary stenting with bare metal stents. Therefore, taken together, shear stress through the activation of the l-arginine nitric oxide pathway seemed to have important beneficial effects in the cardiovascular system.

But how would the endothelium sense such physical forces, and what are the signals to the gene for responding to the forces? There is no doubt that endothelial cells can sense directional flow. Indeed, under physiological conditions the longitudinally shaped cells align in the direction of flow in the vessel wall, thus forming the typical cobblestone pattern. Furthermore, shear forces regulate the expression of many important genes in endothelial cells. Typically, such genes contain shear-stress responsive elements in their promoter region. Interestingly, these binding sites do not resemble the binding motifs of currently known transcription factors.

In this issue of Circulation Research, Cattaruzza et al report that a single-nucleotide polymorphism of the eNOS promoter, the exchange of a cytosine for a thymidine at position –786, is associated with a loss of eNOS messenger RNA and protein expression in endothelial cells, reduced endothelium-dependent relaxations in saphenous veins obtained at surgery from carriers of the gene, and a higher frequency of coronary artery disease as assessed by quantitative angiography.

This is not the first report that unravels functional and clinical consequences of polymorphisms of the eNOS gene. Indeed, several studies have assessed the relation of eNOS polymorphisms on endothelial function, most of them in the human forearm circulation. In one of the forearm studies, the C-786 allele proved to be associated with impaired vasodilation to acetylcholine in patients with hypertension. Similarly, among healthy Japanese subjects, the C-786 allele was associated with blunted cerebral perfusion. Moreover, in Japanese patients with diabetes, the rare variant of the eNOS4 polymorphism (4 a variant) was found to impair flow-mediated vasodilatation in the brachial artery. In the latter studies from Japan, an interaction of the genotypes with smoking could be observed. The G894T polymorphism was associated in several studies with forearm vascular function; however, this was not found in others. Thus, the functional significance of these eNOS polymorphisms remains unclear.

Endothelial function in coronary arteries, on the other hand, has only rarely been investigated. There is one study that examined the relation of eNOS gene variants to endothelial function of the epicardial arteries. Among these, Japanese

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patients referred for chest pain but without coronary artery disease, the C-786 allele was associated with an increased resting tone and an augmented paradoxical vasoconstriction to acetylcholine. In a small statin intervention study assessing cardiac microvascular function, there was no association of the eNOS4 genotype with myocardial perfusion at baseline. Only in the statin intervention arm, adenosine-stimulated flow in subjects carrying the rare eNOS4a allele was significantly increased, conflicting with results of an endothelial dysfunction in the forearm among carriers or this allele. Furthermore, in another study assessing the endothelial function of cardiac resistance vessels, resting tone appeared to be augmented among carriers of the T894 genotype, but not after adenosine stimulation. Finally, in case-control studies among Japanese subjects, those carrying the C-786 and T894 alleles of the eNOS gene had a significantly higher risk for vasospastic angina, supporting a functional role of these polymorphisms on coronary endothelial function.

Given the limited number of studies on the functional and clinical significance of eNOS gene polymorphisms in patients with coronary artery disease, the study of Cattaruzza et al is timely. In particular, because many studies have been performed in Japanese, the importance of the C-786 allele in whites who are much more prone to coronary artery disease remains an important issue. The demonstration of abnormal endothelium-dependent relaxation in isolated saphenous veins from such patients with the C-786 allele provides direct evidence of abnormal vascular function associated with this polymorphism. Although the human saphenous vein reflects only in part endothelium function on the arterial side of the circulation, because veins release considerably less NO than do arteries, the finding is of importance. Furthermore, the authors expand these observations to a cohort of patients who all underwent coronary angiography. In this cohort, they found a frequency of 19% of the C-786 allele in those with angiographic evidence of coronary artery disease, whereas only ~5% without the disease were carriers of the allele. This indicates that a lower level of expression of eNOS, particularly in response to shear forces and, in turn, a reduced bioavailability of NO during episodes of increased activity of the cardiovascular system, is indeed promoting the development of coronary atherosclerosis, as suggested by experimental data. This natural experiment provided by this gene polymorphism, therefore, provides the first evidence that reduced eNOS activation may lead to clinical consequences in the human.

However, whether NO deficiency is also involved in the complications of coronary disease, in particular myocardial infarction, remains uncertain. First, the number of patients included in this study was too small to address this issue. Second, it is possible that plaque rupture involves different genes or gene deficiencies than does plaque development. Nevertheless, the antiplatelet effects of NO, as well as the pronounced modulatory role of NO, on coronary vasoconstriction makes the endogenous nitrate a likely candidate. To expand this hypothesis, however, larger cohorts are urgently needed, allowing for a better assessment on the clinical importance of eNOS polymorphisms in myocardial infarction and cardiac death.

Another finding of the authors may have important clinical implications. They further pursued the question why such a polymorphism of the eNOS promoter would prevent an increased expression of the enzyme under conditions of increased shear and, in turn, increased NO production. Basically, there are two explanations: (1) either the change in the promoter sequence may preclude interaction with a stimulatory transcription factor or, alternatively, binding of an inhibitory element is allowed for by the mutation. With the use of a decoy oligonucleotide mimicking the C-type, but not the T-type, binding motif in the eNOS promoter, Cattaruzza et al demonstrate that shear stress-dependent eNOS expression could be restored. This would suggest that a nuclear inhibitory factor might be involved in the abnormal response of carriers of the C-786 allele. Such an inhibitory binding protein might represent a novel therapeutic target in such patients. Such a therapeutic avenue in the management of coronary artery disease therefore might involve genotyping of the patients and targeted genetic therapy according to the mutations involved. This might provide novel and hitherto unforeseen therapeutic options in the treatment of coronary artery disease.

Panta rei—the Greek philosopher Heraclitus was right: flow is the signal of life. Today we are getting closer to understanding its importance in the human circulation and the pathways involved. In the future, this will allow us to provide better treatment to those in need.

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