Mechanisms of Endothelial Dysfunction Induced by Aging
Role of Arginase I

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Impaired production or biological activity of nitric oxide (NO) released from vascular endothelium is a central mechanism of endothelial dysfunction.1 Large number of published studies demonstrated that endothelial dysfunction is a hallmark of aged endothelium.2 Currently, increased concentration of superoxide anion in vascular wall is considered a major mechanism of endothelial dysfunction caused by aging.3,4,5 Detrimental effect of superoxide anion on aged endothelium is mediated by its chemical reaction with NO leading to inactivation of NO and production of a very potent oxidant, peroxynitrite.5 At the present time, there is no consensus in the literature regarding the exact source(s) of superoxide anion in aged blood vessels. Endothelial enzymes known to be potent generators of superoxide anion include NAD(P)H oxidase, xanthine oxidase, cyclooxygenases, uncoupled endothelial nitric oxide synthases, as well as respiratory chain enzymes in mitochondria.1 Besides increase in superoxide anion production, impairment of endothelial nitric oxide synthase (eNOS) enzymatic activity or reduced antioxidant defense capacity of endothelium may also contribute to elevation of superoxide anion concentration and subsequent endothelial dysfunction.3,4,5

Optimal intracellular level of amino acid L-arginine, a substrate for eNOS, is a critical factor required for normal biosynthesis of NO. Prior studies by Berkowitz and colleagues6 demonstrated that during aging, increased activity of arginase I, (arginase I and arginase II are enzymes that catalyze the hydrolysis of L-arginine to L-ornithine and urea; Figure), may compete for L-arginine with eNOS thereby causing reduced production of NO and endothelial dysfunction. This concept was further supported by the studies demonstrating that in aged arteries genetic inactivation of arginase I could restore NO biosynthesis in vascular endothelium7 leading to the proposal that inhibition of arginase may represent a novel therapeutic strategy in prevention and treatment of endothelial dysfunction induced by aging. Given the fact that aging is a major risk factor for development of cardiovascular disease and that there is no therapies aimed at modulating cardiovascular risk inherent to aging, understanding the mechanisms of aging-induced endothelial dysfunction is of critical importance.

In this issue of Circulation Research, Santhanam and colleagues8 report a series of biochemical, molecular, and functional studies demonstrating that enzymatic activity of arginase I is an important contributor to development of endothelial dysfunction during aging. Presented evidence demonstrate that S-nitrosylation of arginase I cysteine residue 303 stimulates its enzymatic activity by 2 mechanisms: (1) stabilization of the arginase I trimer and (2) by 6-fold reduction of the $K_m$ value of arginase. This, in turn, significantly increases ability of endothelial arginase I to compete with eNOS for L-arginine ultimately leading to impairment of NO biosynthesis in endothelial cells. Most importantly, the authors demonstrate that this phenomenon is present in intact blood vessels isolated from aged rats and that upregulation of inducible nitric oxide synthase (iNOS) enzymatic activity is critical source of NO required for S-nitrosylation of arginase I. The importance of these observations is further underscored by previous reports demonstrating that reduction of intracellular concentration of L-arginine may trigger 2 additional mechanisms that may contribute to endothelial dysfunction in aged blood vessels. First, low intracellular concentration of L-arginine may sensitize endothelial cells to detrimental effect of an endogenous NOS inhibitor N³-asymmetric dimethyl-L-arginine, known to be present in circulating blood under several pathological conditions.9 Second, suboptimal concentration of L-arginine promotes uncoupling of eNOS resulting in formation of reactive oxygen species including superoxide anion, causing further decline of NO availability and consequent deterioration of endothelial function.10

Increased expression of iNOS in endothelium was previously detected in coronary arteries of aged rats.4 This observation has been confirmed in the present study by Santhanam and colleagues.8 Because L-arginine is also a substrate for enzymatic activity of iNOS, activation of arginase I should inhibit production of NO by iNOS. This in turn should prevent S-nitrosylation of arginase I, thereby reducing its activity. However, the nature of this reciprocal regulatory interaction between iNOS and arginase I in intact blood vessels is less clear and remains to be studied. Measurements of NO levels in aged arteries demonstrated significantly reduced levels of NO, suggesting that despite elevated iNOS protein expression availability of NO in vascular wall is reduced by aging. It appears very likely that in endothelium arginase I-induced depletion of L-arginine has inhibitory effect on iNOS, however spatial proximity of iNOS and arginase I in cytosol may explain why even reduced intracellular concentration of NO is still sufficient to cause S-nitrosylation of arginase I.
Is this mechanism of endothelial dysfunction relevant for aging of human cardiovascular system? At the present time, the importance of arginase I in pathogenesis of endothelial dysfunction in humans is not clear and remains to be studied. Existing evidence suggests that L-arginine deficiency may not be critically important for development of endothelial dysfunction in healthy aged subjects. Supplementation of L-arginine does not affect endothelial dysfunction in healthy aged subjects. This is in contrast to supplementation with tetrahydrobiopterin (BH4), an essential cofactor required for enzymatic activity of eNOS. BH4 significantly improves endothelial-dependent vasodilatation in old subjects suggesting that in humans, vascular aging in healthy subjects is associated with loss of BH4 rather than L-arginine. Oxidation of BH4 in human arteries is most likely caused by reported increase in activity of NADPH oxidase and subsequent formation of peroxynitrite. Indeed, peroxynitrite is a very potent oxidant of BH4. However, a recent study demonstrated that arginase plays an important role in loss of NO and pathogenesis of endothelial dysfunction in human blood vessels exposed to proinflammatory conditions in vivo. This observation together with reported propensity of aged blood vessels to express higher levels of proinflammatory cytokines suggest that contribution of arginase to endothelial dysfunction may depend on degree of vascular inflammatory response to aging. Further in vivo studies with selective inhibitors of arginase I, as well as better understanding of the mechanisms involved in regulation of arginase in aging human endothelium, will define importance and therapeutic value of arginase inhibition in prevention and treatment of cardiovascular disease.

Sources of Funding
This work was supported by the National Heart, Lung, and Blood Institute grant HL-53524, and by the Mayo Foundation.

Disclosures
None.

References

Figure. Schematic representation of biochemical pathways involved in catabolism of L-arginine. Both eNOS and arginase use L-arginine as common substrate. Depletion of endothelial L-arginine by increased enzymatic activity of arginase I may inhibit production of nitric oxide leading to endothelial dysfunction. eNOS indicates endothelial nitric oxide synthase.

Key Words: L-arginine ■ nitric oxide ■ S-nitrosylation
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Circ Res. 2007;101:640-641
doi: 10.1161/CIRCRESAHA.107.162701
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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