What We Know and Don’t Know About L-Arginine and NO

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In the latter half of the nineteenth century, amyl nitrate, sodium nitrite, and nitroglycerin were each shown to relieve angina pectoris. These early observations, coupled with the rapid growth of the chemical and pharmaceutical industries, led to the development of nitrovasodilator drugs as the cornerstone of therapy for ischemic heart disease throughout most of the next century. Despite a rather thorough understanding of the pharmacology of these agents, only recently have the vascular actions of nitrovasodilators been placed in their proper biological context. The identification of nitric oxide (NO) as a product of the normal endothelium with smooth-muscle relaxing effects led to the recognition that nitrovasodilators work by providing an exogenous source of NO to the diseased blood vessel. As such, nitrovasodilators may be viewed as replacement therapy for the ailing vasculature.

Over the past 10 years, this paradigm has been strengthened by the observation that the normal endothelium can become dysfunctional when exposed to risk factors for atherothrombosis. In addition, in the setting of many vascular disorders, including essential hypertension and atherosclerosis itself, endothelial dysfunction is apparent. Dysfunctional endothelium is defined by a change in its essential phenotype: the normal endothelial cell promotes vascular smooth muscle cell relaxation, inhibits platelet activation, limits leukocyte adhesion, and inhibits vascular smooth muscle proliferation; the dysfunctional endothelial cell, in the extreme, cannot support smooth muscle relaxation, cannot inhibit platelet activation, is avid for leukocytes, and cannot inhibit vascular smooth muscle cell proliferation. There are varying degrees of endothelial dysfunction, and these may be quantified by different functional assays, including endothelium-dependent vasodilator responses and adhesion molecule expression.

Central to the development of endothelial dysfunction, regardless of its cause, is a loss of bioactive endothelial NO. NO is an important endothelial mediator for each of the principal properties of a normal endothelial cell, and a loss of bioactive NO is associated with endothelial cell dysfunction. The two fundamental mechanisms for the loss of NO bioactivity are reduced synthesis and increased oxidative inactivation by reactive oxygen intermediates. Reactive oxygen species are produced in abundance in those very vascular disorders that are accompanied by endothelial dysfunction. Limiting the generation of reactive oxygen species, especially superoxide, hydrogen peroxide, lipid peroxides, hydroxyl radical, and lipid peroxyl radicals, limits the oxidative inactivation of NO to nitrite, nitrate, peroxynitrite, and lipid peroxynitrites. For this reason, antioxidant therapy has been used in atherothrombosis and hypercholesterolemia to improve endothelial function. Cholesterol-lowering therapy with HMG-CoA reductase inhibitors improves endothelial function, in part by reducing the availability of reactive oxygen species.

An alternative approach to increasing bioactive NO and improving endothelial function is to increase the synthesis of the endogenous nitrovasodilator. Increased synthesis of NO can be achieved by increasing the availability of agonists that stimulate release of NO for the endothelial cell or by providing additional enzyme substrate or cofactors. ACE inhibitors, for example, improve endothelial function by decreasing degradation of bradykinin, an endogenous agonist for NO release from the endothelium.

Another straightforward approach to increasing NO synthesis is to provide additional substrate to the endothelial cell. NO is synthesized in the endothelium by the endothelium-specific isoform of NO synthase, eNOS. The semiessential amino acid L-arginine serves as the principal substrate for the enzyme. First identified in extracts of etiolated lupine seedlings by Schultz and Steiger in 1886, L-arginine was shown to be a product of protein hydrolysis by Hedin nine years later; its structure was not proven until 1910 by Sorenson. While much is known about the intermediary metabolism of L-arginine and its role in nitrogen balance and homeostasis, the importance of this amino acid was heightened by the recent recognition that it serves as the precursor for NO synthesis: NO synthases catalyze the 5-electron oxidation of L-arginine to L-citrulline and produce stoichiometric amounts of NO in the process. Providing supplemental substrate to individuals with inadequate NO, therefore, has been suggested as a rational approach to increasing NO production by the endothelium, and this therapeutic paradigm has met with some success in recent years.

In 1992, Creager and colleagues demonstrated that supplemental L-arginine improves endothelial NO-mediated forearm vasodilator responses in hypercholesterolemic subjects. In the same year, intravenous L-arginine was shown to improve endothelial vasodilator function in atheromatous left anterior descending coronary arteries. Since that time, numerous studies have confirmed that both the acute administration of L-arginine and its chronic oral
administration improve vascular function in hypercholesterolemia, in small-vessel disease, in exercising patients with stable angina pectoris, and at sites of coronary artery stenosis. L-Arginine is believed to evoke these benefits not only by providing eNOS with substrate to enhance NO synthesis, but also by an indirect antioxidant effect, especially in hypercholesterolemia, where its use is accompanied by decreased release of superoxide anion from the endothelium.

Although these effects are consistent and convincing, the precise molecular mechanisms by which L-arginine improves endothelial function remain puzzling. The availability of L-arginine for reaction with eNOS does not appear to be rate limiting; the intracellular levels of the amino acid are in the millimolar range, whereas the enzyme’s $K_m$ for substrate is in the micromolar range. For this reason, other possible explanations have been proposed to explain the effects of L-arginine, and these will be reviewed next.

Investigators have considered several direct mechanisms for the beneficial effects of L-arginine on NO production. Arginine activity is increased in plasma of individuals with vascular disease, and one group has argued that supplemental L-arginine can be used to overcome arginase activity and increase steady-state levels of the amino acid in plasma. L-Arginine is first converted to N$^\gamma$-hydroxy-L-arginine by eNOS, and alternative pathways for N-hydroxylation of L-arginine may increase the availability of this reaction intermediate, which could facilitate substrate turnover by the enzyme. In addition, N$^\gamma$-hydroxy-L-arginine inhibits arginase and may thereby increase intracellular steady-state levels of L-arginine. Clearly, supplemental L-arginine does increase plasma levels of the amino acid, but owing to the great difference between substrate concentration and $K_m$, substrate availability is unlikely to be rate limiting even in the presence of arginase. Another interesting possible mechanism relevant to individuals with atherothrombotic disease is that oxidized LDL and lysophosphatidylcholine may increase intracellular substrate concentration by competitive inhibition of eNOS (by ADMA?), or limited cofactor availability for eNOS. Although this lack of an increase in measurable NO or its metabolites: if NO production is not increased by the treatment regimen, then its bioactivity as measured by flow-mediated brachial artery dilation or cell adhesion molecule expression will also not increase.

Why did L-arginine not increase NO production in this study population? Since serum L-arginine levels increased, the possibilities include either limited cellular uptake, competitive inhibition of eNOS (by ADMA?), or limited cofactor availability for eNOS. In light of the arguments raised above, I remain unconvinced that transport or intracellular substrate concentrations are limiting, even in the presence of established atherothrombotic disease.

Proposed indirect mechanisms by which L-arginine increases bioactive NO in the vasculature are equally diverse. L-Arginine increases insulin secretion, which itself promotes vasodilation. In addition, L-arginine stimulates histamine release from mast cells, which also evokes a vasodilator response. Administered as the hydrochloric acid salt, L-arginine induces an extracellular acidosis that in turn can transiently alter intracellular pH and thereby affect pH-dependent cell-signaling pathways, including calcium transients, that modulate eNOS activity and NO synthesis. Acidic microenvironments can also support the nonenzymatic reduction of nitrite to NO, suggesting yet another mechanism for the benefits of L-arginine hydrochloride. Lastly, L-arginine can attenuate norepinephrine activity, thereby indirectly enhancing the effects of endogenous vasodilators, including NO.

In this issue of Circulation, Blum et al attempt to build on this growing body of evidence for the beneficial effects of supplemental L-arginine by administering oral L-arginine chronically to individuals with established coronary artery disease. In this randomized, double-blind crossover study, the investigators assessed flow-mediated brachial artery dilation and cell adhesion molecule expression before and after one month of therapy. In contrast to prior studies, they failed to find an effect of supplemental L-arginine on these measures of NO bioactivity.

These results, although discordant with prior published data, are not entirely surprising given the speculative basis for the reported benefits of L-arginine in other study groups. There are, in addition, several possible explanations for this outcome that are unique to this study. First and most important, there was no evidence that this dose of supplemental L-arginine actually increased NO production. One previous study reported increases in expired NO gas in individuals given supplemental L-arginine at similar doses. Expired NO was not measured in that study; rather, nitrogen oxides were measured in serum, and no increase was detected compared with placebo treatment. Every other negative result follows from and is consistent with this lack of an increase in measurable NO or its metabolites: if NO production is not increased by the treatment regimen, then its bioactivity as measured by flow-mediated brachial artery dilation or cell adhesion molecule expression will also not increase.

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Why did L-arginine not increase NO production in this study population? Since serum L-arginine levels increased, the possibilities include either limited cellular uptake, competitive inhibition of eNOS (by ADMA?), or limited cofactor availability for eNOS. In light of the arguments raised above, I remain unconvinced that transport or intracellular substrate concentrations are limiting, even in the presence of established atherothrombotic disease. Vascular disease and vascular oxidant stress lead to two additional molecular and biochemical changes in the vascular environment that can modulate NO bioactivity. First, risk factors for atherothrombotic disease are accompanied by limited availability of the critical eNOS cofactor, tetrahydrobiopterin. In the absence of sufficient tetrahydrobiopterin, NO synthase changes its functional profile: instead of oxidizing L-arginine, the enzyme reduces molecular oxygen to superoxide anion, even in the setting of adequate substrate concentrations. The nonspecific antioxidant effects of L-arginine would not overcome this cofactor limitation because only specific synthesis of tetrahydrobiopterin from dihydrobiopterin will suffice, and this requires folate-dependent catalysis by methylenetetrahydrofolate reductase. Second, in atherothrombotic disease, the inducible isoform of NO synthase (iNOS) is expressed
in the atherothrombotic vasculature. This isozyme is much more catalytically active than eNOS and consumes more substrate and cofactors than does eNOS. Furthermore, this isozyme can also serve as a source of superoxide anion in the absence of sufficient substrate or cofactors (specifically, tetrahydrobiopterin). Superoxide flux from iNOS (induced in vascular smooth muscle cells and [microvascular] endothelial cells and present in atherosclerotic plaque) can oxidatively inactivate NO by the mechanisms described above. Thus, the oxidative vascular environment of the atheromatous arteries in these study subjects limits both NO activity and NO production by a variety of potential mechanisms despite adequate L-arginine.

Another possible explanation for the lack of effect of supplemental L-arginine is that the study subjects’ prior drug regimens, on average, improved endothelial function to a degree beyond which further improvement could not be realized. Placebo-treated patients had more than a 6-fold increase in brachial artery flow, and this significant increase in flow was unaffected by L-arginine therapy. In addition, flow-mediated dilation was in the normal range for placebo as well as L-arginine treatment arms. Statin and ACE inhibitor therapy have been shown to improve endothelial function. In addition, β-blockers and aspirin have (weak) antioxidant activity. Thus, optimal improvement in endothelial function may have been achieved by the contemporaneous use of other agents known to improve endothelial function, thereby confounding possible effects of L-arginine itself.

Should L-arginine continue to be investigated as a beneficial agent in the treatment of individuals with endothelial dysfunction? In my view, the answer to this question remains affirmative. The lack of benefit in the present study has several explanations that can and should be investigated in future trials. The absence of a clear explanation for its benefit in prior studies should serve as the basis for future studies designed to address mechanism. The deceptive simplicity of the initial rationale for the use of L-arginine therapy in vascular disease has proven difficult to reconcile with biochemical data, yet its clinical benefits are supported by many studies. More than 75 years passed before the mechanism of action of nitrovasodilators was identified and their relationship to NO recognized. The possible actions of L-arginine are at least as complex, and unraveling its mechanism(s) of action may also require the careful thought and insight that only time permits.

References


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