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Therapeutic Interventions in Endothelial Dysfunction: Endothelium as a Target Organ

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Summary: Endothelial dysfunction is recognized as the initial step in the atherosclerotic process. To date, most interventions attempting to improve endothelial dysfunction have targeted one or more of the numerous risk factors that can cause endothelial damage: hypertension (angiotensin-converting enzyme inhibitors and calcium antagonists), hypercholesterolemia (lipid-lowering agents), cigarette smoking (cessation), sedentary lifestyle (increased physical activity), menopause (estrogen replacement therapy), and diabetes mellitus (control of associated metabolic abnormalities). Interventions targeted specifically to the endothelium remain speculative, as the precise mechanisms of endothelial dysfunction are still being elucidated. Several pharmacologic agents have been suggested to achieve vascular protection through mechanisms that go beyond their primary therapeutic (e.g., hypotensive or hypocholesterolemic) actions; examples of these are angiotensin-converting enzyme inhibitors or HMG-CoA reductase inhibitors. Beneficial changes to the endothelium might result from promotion of vasorelaxation, inhibition of vasoconstriction, reduction in the production of free radicals, or other mechanisms that protect the endothelium from injury.

Key words: atherosclerosis, endothelium, hypercholesterolemia, hypertension, nitric oxide

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Introduction

That a paper can address itself to interventions targeted to the endothelium tells how far we have come since 1980, when Furchgott and Zawadzki reported that acetylcholine-induced vasodilation occurs only in the presence of an intact endothelium.[1] We now recognize that the endothelium-mediated vasodilation observed by Furchgott and Zawadzki is largely due to endothelium-derived nitric oxide (NO), a single molecule with profound effects on cardiovascular physiology. Impairment of endothelial vasodilator function is now established as a major contributor to cardiovascular disease, and accumulating evidence indicates that strategies for restoring endothelial function can have important therapeutic effects.

Risk Factors for Endothelial Dysfunction

Occupying an anatomic position that is both strategic and vulnerable, the endothelium is a target organ for the damaging effects of hypertension, diabetes, and hyperlipidemia, as well as for vascular injuries and mechanical stresses.[2]

Hypercholesterolemia and Atherosclerosis

The possible links between hypercholesterolemia, atherosclerosis, and vascular reactivity began to be examined in the 1980s. Hypercholesterolemia was recognized as a determinant in the pathogenesis of atherosclerosis, and endothelium-mediated relaxation was observed to be impaired in hypercholesterolemic vessels.[3,4]

Hypercholesterolemia enhances the response to vasoconstrictor agonists and attenuates endothelium-dependent relaxation in isolated vessels and in vivo.[5] Reduced activity of endothelium-derived NO in hypercholesterolemic vessels may be an initiating factor in atherogenesis. Endothelium-derived NO is now recognized to inhibit several pathologic processes that are critical to the development of atherosclerosis. These include monocyte adherence and chemotaxis, platelet adherence and aggregation, and vascular smooth muscle proliferation.[6]

Zeiher *et al.*[7] described a progression of endothelial dysfunction in coronary arteries that begins with hypercholesterolemia (Table I). They demonstrated a hierarchy of impairment, with progressive endothelium-mediated alterations in coronary vasomotor tone paralleling the development of early atherosclerosis, culminating in complete loss of endothelium-mediated vasodilation in atherosclerotic coronary arteries. The vasodilatory response to increased blood flow was the last function to be lost, not occurring until myointimal thickening of the arterial wall could be seen on angiography.[7] At least early in the process, endothelial dysfunction is reversible by administration of the NO precursor, L-arginine, to hypercholesterolemic individuals.[6]

Hypertension

Hypertension alters endothelial morphology and function. Platelets and monocytes interact with endothelial cells to a greater degree than in normotensive control vessels,[2] and endothelium-dependent vascular relaxation is reduced.[8]

In a number of earlier studies, antihypertensive therapy was unable to restore normal endothelium-dependent vascular relaxation in resistance vessels in patients with essential hypertension when blood pressure was normalized. The vasodilator response to acetylcholine was blunted even in patients who had received appropriate medical therapy.[9] The endothelial vasodilator dysfunction observed in subjects with essential hypertension appears to be due to a defect in the NO synthase pathway that is not reversible by administration of the NO precursor, L-arginine.[6]

Aging

The effect of aging on endothelium-dependent vasodilation of resistance coronary arteries in humans is characterized by significantly decreased coronary blood flow response to acetylcholine. In contrast, increasing age alters the response to papaverine, a direct smooth muscle dilator, only modestly.[10] Age-related decreases in the production or responsiveness of NO, increases in the production or responsiveness to vasoconstricting factors, or increased degradation of NO in the blood vessel wall may contribute to this effect.[11]

One study[12] of healthy men and women without vascular risk factors indicated that patterns of age-related vascular injury differ according to gender. Loss of flow-mediated dilation correlated with age in both men and women. The decline began in men toward the end of the fourth decade, whereas in women, flow-mediated dilation did not begin to decline until after the early fifties. By the age of 65 years, endothelial dysfunction was apparent in almost all subjects.[12]

Cigarette Smoking

Vasoconstriction,[13] platelet aggregation,[14] and increased monocyte adhesion[15] are but a few of the effects of cigarette smoking that lead to increased risk of atherosclerosis and other cardiovascular diseases. After subjects have smoked cigarettes, there is a doubling in the number of circulating endothelial cells in peripheral blood vessels (presumably reflecting increased turnover and desquamation of the endothelium).[16]

Even young, healthy, light smokers are vulnerable to endothelial damage. Endothelial dysfunction has been reported in the systemic arteries of light smokers beginning with adolescence, and physiologic abnormalities increased with increasing amount and duration of smoking. The threshold for smoking dose and endothelial dysfunction appeared to be ≥20 pack-years.[17] The endothelial vasodilator dysfunction observed in smokers is partially reversible by administration of L-arginine.[6]

Menopause

The Nurses' Health Study cohort[18] provided valuable data on some of the issues involving menopause and cardiovascular risk. Women found at highest risk of coronary heart disease were those who had undergone bilateral oophorectomy without receiving estrogen replacement therapy; those given estrogen replacement after oophorectomy demonstrated no

TABLE I Progression of endothelial dysfunction

Findings on angiography; Stage of atherosclerosis	Hierarchy of impairment
Normal coronary arteries; no risk factors for CAD (controls)	Increased epicardial artery luminal area in response to ACh, sympathetic stimulation, increased coronary flow
Normal coronary arteries; hypercholesterolemia; elevated LDL cholesterol	Selective endothelial dysfunction: vasoconstriction in response to ACh; preserved vasodilation in response to sympathetic stimulation and increased coronary flow
Angiographically normal segment of coronary artery; but disease elsewhere in coronary system	Lost ability to dilate in response to ACh and sympathetic stimulation; flow-dependent dilation intact
Diseased segment of coronary artery	Loss of endothelium-mediated vasoactive functions; vasoconstriction to sympathetic stimulation

Abbreviations: ACh = acetylcholine, CAD = coronary artery disease, LDL = low-density lipoprotein.
Adapted from data in Ref. No. 7.

excess risk, nor did women who had undergone natural menopause.

Menopause, whether natural or surgically induced, was strongly associated with an increased risk of atherosclerosis—that is, detection of calcium deposits in the abdominal aorta—in a study comprising more than 600 women.[19] The risk of atherosclerosis showed an increased trend with the number of postmenopausal years.

Diabetes Mellitus

Vascular disorders are highly prevalent in persons with diabetes and may take several forms: accelerated atherosclerosis, occurring earlier in diabetic patients than in their healthy counterparts and tending to be more severe and more diffuse;[20] thrombosis; hypertension; and hyperlipidemia.[21] The common cellular denominator in this varied pathology may be endothelial cell dysfunction.

Exposure to elevated levels of glucose may contribute to the aberrations of endothelium seen in persons with diabetes.[21] When exposed to increased concentrations of glucose in vitro, rings of isolated normal rabbit aorta are unable to relax normally in response to acetylcholine.[22] Reduced production of NO does not appear to be the cause of the impaired vasorelaxation. Rather, a vasoconstrictor prostaglandin may be elaborated in response to glucose and overcomes the normal vasodilatory effect of NO released by the endothelium. Cyclooxygenase inhibitors restored impaired acetylcholine-induced relaxation in the aortae of diabetic and normal rabbits exposed to elevated glucose in vitro.[21] In humans, the administration of vitamin C improves endothelium-dependent vasodilation, presumably by virtue of its antioxidant effects.[6]

Sedentary Lifestyle

A lack of exercise generally is considered a risk factor for atherosclerosis independent of its negative effects on body weight, blood pressure, and serum lipid values.[11] Chronic immobilization or lack of adequate physical activity, whether by choice or as a result of disease, may be associated with reduced expression of NO synthase and thereby decreased synthesis of NO.[23] So important has physical activity and exercise come to be regarded in maintaining cardiovascular integrity that the American Heart Association has issued a position statement on its benefits.[24] The statement affirms that physical inactivity is a recognized risk factor for coronary artery disease and has been related to increased cardiovascular mortality.

Asymmetric Dimethylarginine

Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor (i.e., antagonist) of NO synthase, reduces the conversion of L-arginine to NO and citrulline. It normally circulates in plasma in humans and is usually excreted unchanged in urine. Elevated levels of ADMA inhibit endothelium-dependent vasodilation, an effect that has been reversed by administration of exogenous L-arginine.[25] Elevated circulating levels of ADMA have been observed in hypercholesterolemic rabbits,[26] in young hypercholesterolemic humans,[27] and in patients with chronic renal failure.[25]

Homocysteine

Elevated levels of homocysteine are associated with premature atherosclerosis. Indeed, nearly one third of persons with premature coronary, carotid, or peripheral arterial disease have elevated plasma levels of homocysteine.[28] Homocysteine may accelerate atherosclerosis by inducing endothelial dysfunction. Infusions of homocysteine have been shown to induce endothelial denudation. In children with homocystinuria (who are at risk for premature atherosclerosis), a dysfunction in endothelial vasodilation can be observed prior to the onset of symptoms of atherosclerosis.[29] Administration of folate (in doses > 800 µcg) is known to reduce homocysteine levels; whether this improves endothelial vasodilator function is under study.

Potential Interventions in Endothelial Dysfunction

With knowledge of endothelial mechanisms and diagnostic methods still evolving, interventions are governed by the manifestations of endothelial dysfunction rather than by the dysfunction per se. Although interventions targeted exclusively at the endothelial monolayer may be developed in the future, some currently available measures have shown promise in improving endothelial dysfunction.

Nonpharmacologic Interventions

Low-cholesterol diet: Cynomolgus monkeys fed a high-fat diet develop hypercholesterolemia and, over time, atherosclerotic lesions similar to those in humans. When placed back on a normal chow diet for several months, vascular lesions regress, with marked reduction in the amount of lipid-laden macrophages in the lesion. Moreover, dietary treatment restored impaired endothelium-dependent vascular relaxation. The mechanism by which endothelium-dependent vascular relaxation was restored by cholesterol lowering is still undefined.[30]

Functional changes and regression of atherosclerosis may occur at different rates and to different degrees in different parts of the vascular bed. Limb blood flow during regression in atherosclerotic arteries of monkeys improved to a greater degree than did hyperresponsiveness of large arteries to serotonin.[31]

Fish oil: It became known in the 1970s that consumption of large quantities of marine fish oils appeared to result in a low incidence of coronary artery disease. Fatty acids in marine fish, particularly cold water fish, differ chemically from those of land animals and those contained in vegetable oils—a greater percentage of marine-derived fatty acids are polyunsaturated, and they are less vulnerable to oxidation. Eicosapentaenoic acid and docosahexaenoic acid in marine lipids can substitute for arachidonic acid. Like arachidonic acid, they can

be converted into an active form of prostacyclin (a vasodilator and inhibitor of platelet aggregation). Unlike arachidonic acid, they are converted into an inactive form of thromboxane (the vasoconstrictor and platelet agonist). Therefore, these omega-3 fatty acids shift the balance in the arachidonic acid cascade to the side of the vasodilator/platelet antagonist prostacyclins. Some effects attributed to marine fish oils included lowered levels of triglycerides, total cholesterol, and very-low-density lipoprotein cholesterol; reduced platelet aggregation; and prolonged bleeding time.[32] Monkeys fed an atherogenic diet with half the fat-derived calories from fish oil showed evidence of reduced superoxide anion production in coronary artery endothelium after 1 h of ischemia and 2 h of reperfusion.[33] Swine fed a high-fat diet develop an endothelial vasodilator dysfunction that is reversible by treatment with fish oil.[6]

Exercise: According to a study of patients whose physical activity was limited by congestive heart failure, flow-dependent dilation can be enhanced by physical training. After 4 weeks of hand-grip training, flow-dependent dilation was restored, most likely by increased endothelial release of NO. The effect of physical training was local, however, being limited to the trained arm, and lasted for only 6 weeks.[23]

Smoking cessation: The improvements in vascular function that follow cessation of cigarette smoking partially reverses the adverse effects of cigarette smoking on the vasculature. Endothelial dysfunction improves with smoking cessation. Flow-mediated dilation was observed to be better in male former smokers than in current smokers, albeit impaired in both groups.[17]

The lipid profile also benefits from smoking cessation: high-density lipoprotein (HDL) cholesterol and apolipoprotein A-1 increase, whereas triglycerides decrease. An increase in lipoprotein lipase correlated significantly with the increase in HDL cholesterol.[34] Moreover, the increased risk of myocardial infarction conferred by smoking decreases to the level of men who never smoked within a few years after tobacco cessation.[35]

Antioxidant supplements: Because oxidation of low-density lipoprotein (LDL) cholesterol contributes to endothelial dysfunction, investigators have reasoned that a diet rich in antioxidants may be protective.[2] Results of clinical studies have not consistently shown a benefit, however. In one trial of hypercholesterolemic patients, 1 month of treatment with relatively high doses of beta-carotene and vitamin C and E supplements delayed the onset of oxidation of LDL and decreased the maximal rate of LDL oxidation, but endothelial function was still impaired.[36] Nonvitamin antioxidants, antioxidant enzymes, or concomitant reduction in LDL levels may be required to improve endothelium-dependent vasodilation in hypercholesterolemic patients.

Other investigators reported that vitamin C reversed endothelial dysfunction in the brachial circulation of patients with coronary artery disease. In a placebo-controlled, blinded study,[37] oral administration of 2 g of ascorbic acid restored endothelium-dependent vasodilation.

L-Arginine supplementation: With the recognition of NO as the major mediator of endothelium-dependent relaxation, interest began to center on L-arginine, the precursor of NO. Investigators hypothesized that increasing the availability of L-arginine might enhance synthesis of NO and thereby promote vasodilation.[5]

The first evidence that L-arginine might have an antiatherogenic effect came from a study of hypercholesterolemic rabbits whose diet was supplemented with an average sixfold increase in daily L-arginine intake.[38] Compared with lesions of hypercholesterolemic controls, atheromatous lesions in the thoracic aortae of the L-arginine-supplemented animals had markedly decreased surface area and reduced intimal thickness. Endothelium-dependent relaxation improved, even though the supplemented diet did not affect the animals' serum cholesterol levels.[38]

The potential benefits of L-arginine following arterial injury were studied 4 weeks after the iliac arteries of normocholesterolemic rabbits were denuded by a balloon catheter.[39] Administration of L-arginine during the 4-week period reduced neointimal thickening and improved acetylcholine-induced vasorelaxation. The similarity of this model to restenosis after percutaneous transluminal coronary angioplasty marks it for particular interest.

Coronary artery dimensions and blood flow in hypercholesterolemic patients and normocholesterolemic controls were compared before and after L-arginine infusion. L -arginine augmented endothelium-dependent dilatation in the coronary microcirculation of hypercholesterolemic patients who had shown impaired endothelium-dependent dilatation. No effect was observed in the normocholesterolemic controls.[40]

Essential hypertension may be a setting in which L-arginine supplementation cannot mitigate pathologic changes. Patients with essential hypertension and diminished acetylcholine-induced vasodilation did not respond with augmented endothelium-dependent vasodilation to increased availability of NO substrate.[41]

Pharmacologic Interventions

Several categories of drug used to treat cardiovascular disease have proven to ameliorate impaired endothelial vasodilation (Table II).

Lipid-lowering agents: Cholesterol-lowering therapy has been associated with a decreased risk of ischemic coronary events even in the absence of angiographic regression of atherosclerosis. Restoring coronary endothelial function may be more important to improved clinical outcome than reducing the degree of stenosis.[42]

Reversal of coronary endothelial dysfunction in patients with symptomatic coronary atherosclerosis predates changes in vascular structure. Treatment with lovastatin does not improve coronary artery endothelial responses to acetylcholine after 12 days, but does significantly improve epicardial coronary artery responses to acetylcholine at 5 1/2 months.[42] A recent report indicates that endothelial vasodilator function is improved immediately after plasmapheresis in patients with familial hypercholesterolemia.[43]

TABLE II Current agents that can reverse endothelial dysfunction

Pharmacologic strategy	Examples
Lipid-lowering agents	HMG-CoA reductase inhibitors, cholestyramine
Inhibitors of renin-angiotensin system	ACE inhibitors, angiotensin II receptor antagonists
Calcium-channel blockers	Verapamil, nifedipine
Antioxidants	Vitamin C, vitamin E
Enhancement of NO synthase pathway	Folate, arginine, estrogen
Cytoprotective agents	Superoxide dismutase, probucol
Substitutes for protective endothelial substances	Nitrovasodilators; analogs of prostacyclin

Abbreviations: ACE = angiotensin-converting enzyme, HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A, NO = nitric oxide.
Adapted from data in Ref. No. 13.

Reduction of LDL cholesterol alone failed to reverse endothelial dysfunction in coronary arteries in another study,[44] but the impairment was significantly improved when antioxidant therapy was added to the regimen. Improvement in vasomotor response to acetylcholine was significantly greater in the combined therapy (lovastatin and probucol) group than with diet or LDL cholesterol lowering alone.

Angiotensin-converting enzyme (ACE) inhibitors: The role of the renin-angiotensin system in endothelial dysfunction relates primarily to angiotensin II as a potent endothelium-derived contracting factor. Angiotensin II, the vasoactive product of angiotensin I, is produced by the action of ACE. Although this reaction takes place primarily in the lung, a tissue ACE system has also been found in endothelial cells throughout the vasculature.[13]

The vasoconstrictive effect of tissue ACE in generating angiotensin II is normally balanced by the effects of NO and prostacyclin. When the endothelium is damaged or dysfunctional, however, the countervailing effects of these endothelial vasodilators are lessened.[45]

One of the first studies to demonstrate an improvement in endothelial dysfunction with an antihypertensive agent was the Trial on Reversing ENdothelial Dysfunction (TREND).[45] TREND was conducted in 129 normotensive (or controlled hypertensive) patients with coronary artery disease to determine whether treatment with an ACE inhibitor (quinapril 40 g daily) could improve endothelial dysfunction. Angiograms performed at baseline and at 6-month follow-up showed significant improvements in endothelial vasomotor function (assessed by response to acetylcholine) in the quinapril-treated patients.

The beneficial mechanisms of quinapril in this 6-month trial probably relate to the effects of ACE inhibition on both angiotensin II and bradykinin, which is a potent vasodilator. Angiotensin-converting enzyme inhibition of angiotensin II counters its contractile effect on smooth muscle and reduces the generation of superoxide anions. In diminishing the breakdown of bradykinin, ACE inhibition enhances the bradykinin-induced release of NO by endothelial cells. In the TREND study, quinapril improved endothelial dysfunction without altering lipids or reducing blood pressure.[45]

Calcium-channel blockers: Cholesterol-fed rabbits were given a calcium antagonist at a dose too low for an antihypertensive effect. Treated rabbits had less impairment in endothelium-dependent cholinergic relaxation than untreated but hypercholesterolemic controls. Thus, treatment with a dihydropyridine calcium-channel blocker inhibited atherogenesis to a partial degree in these animals without reducing arterial blood pressure.[3] In humans, several trials of calcium-channel blockers have been concordant in showing an effect of these drugs in inhibiting the development of new lesions; however, there is no evidence that calcium-channel blockers modify existing lesions or reduce coronary events.

Estrogen replacement: Although the benefits of estrogen replacement therapy after menopause include an improved lipid profile, multiple regression analyses have indicated that only 25 to 50% of the reduction in cardiovascular events can be attributed to lipid-lowering effects.[46] The finding that estrogen receptors are localized on endothelial and smooth muscle cells of several mammalian species has suggested that the hormone may directly influence vascular function.[47, 48] More recently, estrogen receptor expression was demonstrated in human endothelial cells, suggesting that estrogen may act directly on human vascular tissue.[49]

These findings prompted several studies. For example, a trial[50] of estrogen administration in postmenopausal women with atherosclerotic coronary arteries and mild hypercholesterolemia found that estrogen improved endothelium-dependent vasodilation without any effect on lipids. After 9 weeks of estradiol therapy (1 or 2 mg/day), flow-mediated vasodilation in the brachial artery was improved. The effects of estrogen to enhance endothelial vasodilator function may be due to an antioxidant effect, or to an estrogen-induced enhancement of NO synthase expression.

Future Therapeutic Possibilities

Strategies specifically targeted to restoration of endothelial function may be expected to reverse or reduce the progression of vascular disease and to normalize vascular reactivity. A mechanistic understanding of the pathophysiology of endothelial dysfunction is required for such specific therapies to be developed. With respect to derangements of the NO synthase pathway, a number of possible mechanisms merit exploration. Reduction in the availability of the precursor, or alterations in the enzyme NO synthase, may explain the beneficial effects on

NO elaboration of supplemental L-arginine or folate (which is a precursor of tetrahydrobiopterin, a cofactor for NO synthase). Alternatively, elevated activity of α-methylase I or reduced activity of dimethylarginine dimethylaminohydrolase may explain the increased circulating levels of ADMA observed in patients with vascular disease. Observations by our group and others indicate that ADMA is an endogenous modulator of NO synthase. Finally, the expression (as well as the activity) of NO synthase can be modulated. For example, estrogen is known to increase the transcription of NO synthase, indicating proof of concept for another therapeutic strategy worth exploring.

Conclusions

Improved endothelial function appears to be possible via a variety of currently available methods, with novel approaches still to come. It seems reasonable to expect that future therapeutic strategies and agents will be directly targeted to this monolayer of cells that regulates vascular tone and structure. Early detection of endothelial dysfunction may be a useful measure to guide therapy prior to the development of symptomatic atherosclerosis.

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