E-mail stephen.epstein@medstar.net

From the Cardiovascular Research Institute, MedStar Health Research Institute, Washington, DC (S.E.E., R.M.L.-S., M.S.B.), and Department of Physiology and McAllister Heart Institute, University of North Carolina, Chapel Hill, NC (J.E.F.).

Correspondence to Stephen E. Epstein, MD, Cardiovascular Research Institute, Washington Hospital Center, 110 Irving St, Washington, DC 20010.

E-mail stephen.epstein@medstar.net

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polymorphisms responsible for these differences in mice have not yet been identified. However, genetically targeted differences in expression of Vegfa and Clic4 have dramatic effects on collateral formation in the embryo and maturation in the neonate. Moreover, genetic-mapping studies in mice identified a major quantitative trait locus on chromosome 7 and several additional loci that together account for >70% of the natural variation in collateral extent. (The genes or genetic elements underlying these quantitative trait locus, however, have not yet been identified.) Genetic influences also affect the capacity of collaterals to remodel. Interestingly, genetic variation in collateral remodeling maps to the same chromosome 7 quantitative trait locus controlling native collateral diameter—with correlation analysis supporting a major role of initial diameter and thus shear stress—and to a novel locus independent of collateral diameter on chromosome 11, as well.

It was generally assumed that collaterals develop in response to arterial obstruction. However, along with the findings that genetics importantly contribute to collateral phenotype, it is now clear that collaterals form in mice late during embryogenesis after the arterial-venous circulation is formed, followed by early postnatal pruning, increase in diameter, and acquisition of smooth muscle cells, which by several weeks after birth establishes the number and diameter of collaterals present in the young adult. The presence of a native collateral circulation is also inferred from earlier studies comparing presumed collateral-dependent flow (after ligation of the left anterior descending coronary artery) among different mammalian species. These studies also demonstrated the functional importance of these native collaterals and the influence of genetic background; acute occlusion of a major artery to brain or hindlimb resulted in less tissue injury in strains having greater native collateral extent, with genetic differences accounting for the majority of the variability in collateral extent and infarct volume.

An intriguing question is whether the collateral circulation interconnecting adjacent arterial trees within the microcirculation has a physiological function in the absence of arterial obstructive disease. Although the answer is not known, it seems likely that it does. We speculate that the presence of such vessels provides a second source of blood flow from adjacent arterial trees, in addition to the trunk of the tree supplying a given region, to optimize local metabolic vascular regulation, ie, to help match oxygen supply to oxygen demand under varying levels of regional tissue metabolism.

The conclusion that collaterals are native to the organism and their presence is not dependent on arterial obstruction has human relevance. In a small group of patients with normal coronary arteries undergoing aortic valve replacement, retrograde flow, measured distal to an obstructing intracoronary balloon, was taken as an index of collateral conductance. Collateral flow was present in all patients, and about one-third had values falling in the range of patients with demonstrated coronary artery disease. Similarly, Seiler and co-workers found that collaterals were present in individuals without angiographically demonstrable stenotic coronary arteries. Although collateral function was highly variable in these individuals, one-fifth had, immediately after balloon-induced coronary artery occlusion, collateral flow sufficient to prevent myocardial ischemia. Thus, collaterals are present in humans and other mammals before arterial obstructive disease develops, and there is wide variation in the functional capacity of such collaterals. Given the data in mice indicating strong genetic-related determinants of collateral number, diameter, and remodeling, we speculate that differences in native collateral function found in humans also reflect, at least in part, gene variants that function within signaling pathways specifying formation of the native collateral circulation and its remodeling following occlusion.

Effects of Aging on the Collateral Circulation
It was first demonstrated >10 years ago, and subsequently confirmed, that aging decreases collateral-dependent flow recovery following acute arterial obstruction (Figure 1). Just as there are multiple processes involved in augmenting collateral-dependent flow (Figure 2), so too there are multiple changes that occur during aging that impair each of these processes, thereby interfering with the capacity of collaterals to compensate for impaired flow following arterial occlusion. The following discussion details several of these aging-induced changes.
Aging-Induced Collateral Rarefaction and Decreased Collateral Diameter

Investigation of mechanisms responsible for aging-induced impaired collateral flow recovery demonstrated in the mouse hindlimb that aging causes “age-dose-dependent” collateral rarefaction (ie, decreased collateral number) and diminished collateral diameter. These changes, as well as increased collateral tortuosity, also occurred in the cerebral circulation with aging, a finding demonstrated previously in aged rats and cats. Such alterations in the cerebral circulation resulted in a 6-fold increase in calculated collateral resistance, and were associated with increased stroke severity. Rarefaction was not accompanied by loss of arterioles or capillaries in the general cerebral circulation.

Effects of Aging on Collateral Remodeling

Normal Processes Contributing to Remodeling

The major mechanism initiating collateral remodeling consequent to arterial obstruction is the increased shear stress imposed on collateral endothelium. Before arterial obstruction, native collaterals interconnecting adjacent arterial trees are small in diameter (≈20–40 μm) with high resistance. Under resting conditions, no net pressure gradient exists across them, as shown by collateral flow being near zero and weakly oscillatory. Arterial obstruction reduces downstream pressure, causing a pressure gradient to develop across the interconnecting collateral network, with associated increased flow and increased shear stress. The increased shear stress augments expression of adhesion molecules, chemokines, cytokines, vasoactive molecules, and proinflammatory genes. These changes, along with a systemic response evoked by tissue ischemia, result in both mobilization of stem, progenitor, and inflammatory cells (most importantly monocytes), and their homing to the “injured” tissue and perivascular area of native collaterals. Secretion of cytokines by these cells produces a positive feedback loop, all contributing to enhanced remodeling.

Deleterious Effects of Aging on Collateral Remodeling

Although previous studies on the effects of aging on vascular remodeling in animals focused on arteries rather than collaterals, more recent studies have measured collateral diameter both in the mouse hindlimb and brain before and several days after acute occlusion of the femoral or middle cerebral artery, respectively. Aging reduced collateral remodeling in both vascular beds. Although studies in humans demonstrate both age-related arterial abnormalities, including decreases in arterial remodeling, and age-related decrease in collateral function, there are no studies in humans that provide insight into the specific effects of aging on collateral remodeling.

Several observations indicate aging-induced impaired collateral remodeling is multifactorial: (a) aging impairs endothelial nitric oxide synthase (eNOS) pathways in collaterals and shear stress pathways (Figure 3 and following text) in mesenteric arteries and in the hindlimb; (b) aging also increases oxidative stress in the aorta, coronary arterioles, and mesenteric arteries, and decreases expression of hypoxia-inducible factor-1 and vascular endothelial growth factor. Each of these changes, if also present in the collateral circulation (as is the case with aging-induced eNOS changes), could impair collateral remodeling and collateral blood flow recovery; (c) aging compromises mobilization and homing of stem, progenitor, and inflammatory cells to the perivascular region of remodeling collaterals, processes normally occurring after arterial occlusion. Fewer cells homing to the region of growing collaterals means the collaterals will be exposed to fewer secreted cytokines and growth factors that would ordinarily contribute to their remodeling; (d) aging reduces the capacity of stem cells to secrete cytokines involved in remodeling; (e) aging-related increased arterial stiffness, mediated in part by increased collagen content and alterations of extracellular matrix, if also relevant to collaterals, would provide another mechanism impeding collateral remodeling; (f) reduced vasodilation caused by aging-related endothelial
dysfunction also probably contributes to impaired collateral remodeling, given that collateral remodeling is reduced in cerebral and hindlimb collaterals in eNOS-deficient mice. Each of these aging-induced abnormalities in remodeling mechanisms could independently cause reduced remodeling. However, it is likely that impaired remodeling results from the simultaneous effects of aging on a multiplicity of these interrelated and interactive mechanisms and pathways (Figure 4).

Aging-Induced eNOS Dysfunction and Increased Oxidative Stress: Their Interactions and Potential Effects on the Collateral Circulation

Normal eNOS Signaling Pathway
The normal eNOS signaling pathway is complex (Figure 3). The generation of nitric oxide (NO) derives from activities of the catalytic domains of coupled eNOS, including a flavin-containing NADPH-binding reductase and a heme-
binding oxygenase. These domains contain binding sites for the redox-labile cofactor tetrahydrirobipterin (BH4) and the eNOS substrate t-arginine. Normally, on eNOS activation (phosphorylation of Ser-1177), electrons flow from NADPH through the reductase to the oxygenase domain. This converts t-arginine to citrulline resulting in NO production, a process requiring fully reduced BH4. Whereas endothelial cells (ECs) produce NO, major NO-induced vascular effects occur after NO enters neighboring smooth muscle cells (SMCs), where it increases cGMP, leading to vasodilation through phosphorylated vasodilator-stimulated phosphoprotein. NO also has antioxidant and antiapoptotic effects. It is not clear at this time which eNOS/NO activities are mediated via, or are independent of, cGMP other than vasodilator and antioxidant activities.

Aging-Related Molecular Changes in eNOS Signaling Pathways
Aging deleteriously alters various components of the eNOS-signaling pathway. Thus, in mice, aging not only decreases eNOS protein levels in calf muscle under nonischemic conditions, but it also decreases Ser-1177 phosphorylation. Phosphorylation at Ser-1177 is necessary for eNOS activation and signaling. Importantly, impaired eNOS signaling has also been demonstrated in collaterals: immunopositive phospho-eNOS is less in gracilis collaterals of old versus young mice. The functional relevance of this is confirmed by the finding that aging is also associated with reduced levels of a critical downstream product of NO signaling, phosphorylated vasodilator-stimulated phosphoprotein.

Aging-Related Changes in Oxidative Stress
Aging increases reactive oxygen species, a change that has major deleterious effects on normal functioning of the eNOS pathway. Among the effects of increased oxidative stress is oxidation of BH4, leading to formation of eNOS monomers. Loss of the eNOS dimer architecture causes uncoupling of electron flow (uncoupling of eNOS), resulting in superoxide rather than NO production (Figure 3). Thus, Akt-mediated eNOS phosphorylation, which normally enhances NO production, increases eNOS-derived superoxide rather than NO when eNOS is uncoupled. Superoxide transforms any NO produced into peroxynitrite, which further reduces availability of NO and further oxidizes BH4. The presence of increased oxidants, in part through oxidation of BH4 and in part owing to conversion of eNOS to a monomer, is one of the key mechanisms responsible for the switch of eNOS from an NO to an O2- generator. This switch transforms eNOS from a vascular-protective enzyme into an enzyme that probably is a major contributor to aging-induced dysfunction of the collateral circulation. These dysfunctional eNOS pathways and the effects they produce are depicted in red in Figure 3.

Deleterious Functional Effects of Aging-Related Changes in the eNOS-Signaling Pathway and in Oxidative Stress
Impaired endothelial release of NO produces the commonly recognized clinical marker of endothelial dysfunction—reduced vasodilation in response to stimuli that increase vascular wall shear stress. This reduced vasodilator activity may contribute to the anatomically smaller diameter collaterals observed in older animals. However, increased oxidative stress and dysfunctional eNOS signaling have a much broader range of effect than just impairing vasodilator function. That the eNOS/NO pathway exerts antiapoptotic effects, and that aging causes dysfunction of the pathway and also causes collateral rarefaction, suggests the possibility that ECs and/or SMCs derived from old mice might be more sensitive to apoptotic stimuli, a situation that could predispose to dropout of the vessels they line. Indeed, both ECs and SMCs derived from old mice and tested in vitro are more sensitive to apoptosis induction. Furthermore, when ECs are preincubated with sodium nitroprusside, an NO donor, the apoptotic responses are attenuated. These findings are supported by Hoffmann et al who demonstrated that late-passaged ECs (not, however, derived from old mice) exhibited increased sensitivity to an apoptotic stimulus compared to early passaged cells, an effect attenuated by exogenous NO. SMCs derived from old mice also are more sensitive to an apoptotic stimulus, and, importantly, the amount of apoptosis is attenuated by preincubating the cells with tadalafil, a phosphodiesterase-5 (PDE-5) inhibitor that reduces breakdown of the critically important downstream product of NO signaling, cyclic GMP.

Interestingly, an actual primary role of PDE-5 in contributing to the deleterious effects of aging on the eNOS/NO system and on the collateral circulation was suggested by the finding that PDE-5 expression is increased in EC cells derived from old versus young mice. Increased PDE-5 activity would result in reduced levels of cyclic GMP in cells, thereby possibly contributing to increased oxidative stress and increased sensitivity to apoptosis, as well as to decreased collateral remodeling. Additional importance attaches to this observation given that PDE-5 inhibitors increase the eNOS dimer/monomer ratio (Figure 4) and decrease oxidative stress. Genetic studies provide evidence consistent with the concept suggested by these associative studies, namely, that there is a causal relation between intact eNOS signaling and collateral viability. Thus, eNOS KO mice, while having normal collateral number at birth, exhibit rapid collateral dropout over the first 3 months of life that is worse 6 months later. The data, considered in total, indicate that the eNOS system is necessary for maintenance of the collateral circulation during natural aging and that aging-induced dysfunction of this pathway significantly contributes to collateral rarefaction and to impaired collateral remodeling. Such deleterious changes in eNOS activities can occur as a primary aging-induced epigenetic change, such as methylation of sites on the eNOS gene or on genes encoding molecules in the eNOS signaling pathway, resulting in altered gene expression. Impaired eNOS activity could also derive from altered activity of microRNAs (miRNAs) that influence expression of the protein products of these genes, as discussed below, or through the aging-related increase in oxidative stress, which would exert important functional effects on eNOS activities through various mechanisms (Figure 3).
Speculations on Potential Therapeutic Strategies Deriving From the Effects of Aging on eNOS Pathways and Oxidative Stress

The aging-relating effects on eNOS pathways and on oxidative stress raise interesting possibilities relating to potential therapeutic strategies. For example, the aging-induced dysfunctional eNOS pathway is accompanied by increased PDE-5 expression. Moreover, sildenafil, a PDE-5 inhibitor, can reverse abnormalities in NO bioavailability, oxidative stress, and eNOS dimer to monomer switch. It is therefore possible that PDE-5 inhibitors can reduce the aging-related collateral dysfunction that is mediated by a dysfunctional eNOS pathway and increased oxidative stress. Another potential therapeutic-related approach might involve targeting the increased oxidative stress occurring with aging. Thus, Miller and coworkers examined the capacity of the ileal collateral dysfunction that is mediated by a dysfunctional eNOS pathway and increased oxidative stress. Another potential therapeutic-related approach might involve targeting the increased oxidative stress occurring with aging. Thus, Miller and coworkers examined the capacity of the ileal artery of aged rats to remodel, in vivo, in response to ligation of adjacent arteries. They found that luminal expansion was absent in aged rats, but that expansion was restored by antioxidant treatment. These beneficial effects were abolished by coadministration of N^6-nitro-l-arginine methyl ester (which inhibits eNOS conversion of l-arginine to l-citrulline, with resulting decreased NO formation). The investigators therefore posited that the aging-associated impaired remodeling they demonstrated was caused by a dysfunctional eNOS system induced by increased oxidative stress. These results, along with the data summarized above, suggest that antioxidants may exert beneficial effects on some of the deleterious effects aging exerts on the collateral circulation. These speculative concepts relating to targeted therapeutic strategies are deserving of further investigation.

miRNA Modulatory Activity as a Potential Mechanism for Aging-Induced Changes in Collateral Function

At a more fundamental level, what are the major underlying mechanisms likely responsible for these complex age-related changes in eNOS and collateral functionality? Aging-induced changes in miRNA levels may represent 1 such mechanism. miRNAs are short noncoding RNAs that bind to messenger RNA transcripts and thereby inhibit gene expression. Although no studies have as yet been reported demonstrating that any of the effects of aging on the collateral system are mediated through aging-induced miRNA alterations, we believe this will be a fruitful area of future research. For example, the levels of certain miRNAs are altered by aging, and miRNAs modulate genes involved in eNOS activity. Because, as noted above, (a) eNOS is critical for collateral development and maintenance, and (b) eNOS activity in collaterals is decreased by aging, it is possible that aging causes some of its changes in collateral phenotype and function through alterations of miRNAs regulating eNOS activity.

Other possible miRNA targets for aging, which might provide additional molecular mechanisms by which aging impairs collateral function, include (a) miRNAs that modulate genes involved in reactive oxygen species and apoptosis pathways, and (b) miRNAs (such as miR-16, 424, miR-126, miR-21, miR-34a, miR-9) that appear involved in angiogenesis, inflammation, and/or vascular endothelial growth factor or fibroblast growth factor expression; the inhibition of which reduces ischemia-induced tissue damage in mouse models of hindlimb ischemia and myocardial infarction; and, when overexpressed in ECs, increases Akt and eNOS phosphorylation, increases NO production, and decreases apoptosis.

Aging-Induced Impairment of Bone Marrow Progenitor Cell Function, Mobilization, and Homing to Developing Collaterals

Mobilization of progenitor and mononuclear cells from bone marrow and their homing to the perivascular region of collaterals within hours after acute occlusion appear to contribute to collateral remodeling. processes compromised by aging. Moreover, aging impairs progenitor/stem cell function, further compromising the facilitatory role played by bone marrow-derived progenitor cells in arteriogenesis.

An example of a mechanism contributing to impaired mobilization is found in the CXCR4/stromal cell-derived factor-1 binding interaction between stem cells and their niche-binding receptors. The membrane-bound extracellular peptidase CD26 (dipeptidyl peptidase-4), expressed on many hematopoietic cells including stem and progenitor cells, cleaves certain dipeptides from the N terminus of polypeptide chains. One of its substrates is stromal cell-derived factor-1; when this is cleaved by CD26 the chemoattractant properties of stromal cell-derived factor-1 for CXCR4 are blocked, thereby abolishing theanchoring properties exerted by the bond created between stromal cell-derived factor-1 and CXCR4-expressing cells. Consequently, such cells are released from bone marrow and into the circulation. Of note, old mice have decreased bone marrow levels of CD26 mRNA under baseline conditions and significantly fewer CD26-positive cells 2 hours after hindlimb ligation, findings compatible with impaired CD26-mediated mobilization.

To ascertain whether the aging-related impaired mobilization was a specific defect of the bone marrow tissue, or was due to a systemic effect that suppressed mobilization, a “rescue experiment” was performed. Could mobilization and homing improve in old mice after bone marrow (BM) cell transplantation from young donors? When BM obtained from young mice was transplanted into old mice, mobilization and homing to collaterals undergoing remodeling improved, indicating that a specific defect of the BM and BM cells of old mice compromises these functions.

Aging-Induced Impaired Responsiveness of Preexisting Collaterals

Unfortunately, despite greater homing of cells to ischemic tissue when young BM is transplanted into old mice, blood flow recovery failed to improve. These results suggest that aging impairs the responsiveness of collaterals to stimuli that promote collateral remodeling. This conclusion is supported by results obtained by use of a surgical skin flap model of ischemia. The investigators found that, when young BM is transplanted into aged hosts, recovery of flap perfusion was identical to that of aged controls.
Impaired target tissue responsiveness is supported by an additional experiment. Based on the critical role of EC proliferation in both ischemic angiogenesis and collateral remodeling, a study was performed to determine whether aging impaired the angiogenic response of ECs. In a Matrigel plug assay, in which plugs were impregnated with the potent angiogenic-inducing growth factor basic fibroblast growth factor and implanted subcutaneously into the abdominal wall of mice, aging reduced the capacity of ECs to respond to the angiogenic agent, ie, fewer ECs migrated into the plug, and those that did were deficient in forming blood vessels.

Effects of Aging on the Collateral Circulation of Humans

Definitive data on the effects of aging on collaterals in humans are very limited. This is in part because of the difficulty of measuring collaterals or collateral-dependent flow in humans and, in part, because, if measured, it is invariably in patients with coronary artery disease. Collateral function in these patients is critically influenced by the degree of coronary obstruction and the time during which the obstruction has been present, making it impossible to differentiate rarefaction from remodeling. Thus, any age correlation would be overwhelmed by these factors unless very large numbers of patients are studied so that statistical methods could dissect out independent associations.

This was the case in 1 article in which investigators studied by angiography almost 2000 patients with a totally occluded infract-related artery within 72 hours of the acute myocardial infarction. Collateral function was assessed by Rentrop scoring. The authors concluded that advanced age was an independent factor predicting impaired collateral circulation.

In addition, Seiler and colleagues measured collateral function in a group of individuals without significant coronary artery disease, thereby avoiding the limitations inherent in assessing patients with coronary artery disease. However, the limited number of individuals studied, in conjunction with the limited age range, made it impossible to draw definitive conclusions about the effects of age on collateral number or function.

Finally, other studies in humans, although not including analysis of either aging or of collaterals, have demonstrated that various risk factors (such as low birth weight, prematurity, hypertension, obesity, and diabetes mellitus) are associated with abnormal function of the microcirculation (vessels <150 μm in diameter), including vascular rarefaction.56 Such results at least suggest that our preceding discussion on the effects of aging on collateral function, although focusing on animal and in vitro studies, likely applies to humans.

Summary of the Functional Consequences of Aging-Induced Collateral Dysfunction

Aging compromises many of the processes (primarily those contributing to impaired remodeling and an actual dropout of collaterals) that influence the capacity of collaterals to restore perfusion following occlusion of a major conduit artery. It is therefore not surprising, as noted, that in old versus young mice, occlusion of the major conduit artery of the hindlimb results in increased hindlimb tissue injury and decreased hindlimb function, and in the brain results in a 3-fold increase in severity of infarct volume.17-20 Although the studies performed in experimental animals leading to these conclusions have no parallel human studies with which to compare, it seems likely that the formidable deleterious effects aging exerts on the collateral circulation of animals has human relevance.

Conclusions

Aging compromises native collateral extent and collateral remodeling such that flow recovery following arterial occlusion is significantly reduced in multiple vascular beds. Of great concern from a clinical therapeutic perspective is that the responsible causes are multiple. These multiple levels of dysfunction, if they also occur in humans, may have played a role in the so-far inconsistent results of existing randomized clinical trials testing therapeutic approaches to enhance collateral function. These considerations suggest that aging conveys a certain level of resistance to therapeutic intervention. Further, if the aging-induced collateral rarefaction that occurs in mice also occurs in patients, the possibility arises that current strategies designed to enhance collateral function may be severely limited because the primary therapeutic target, the extent of the native preexisting collateral circulation, is diminished. Another important unknown that has clinical/therapeutic implications is the time line over which aging-induced deterioration in collateral function occurs: does deterioration occur gradually over an individual’s life, or does it occur relatively quickly and is biologically important only in the later decades of life?

These considerations raise the question as to whether modifications of existing strategies to enhance collateral function can at least be modestly successful, or whether alternative approaches based on a preventive approach will be required. At the least, the issues addressed in this review indicate that innovative novel approaches to preventing aging and risk factor-induced collateral dysfunction are necessary. The successful development of such approaches will require expanding our still-limited knowledge of the genetic determinants of collateral development and, in all probability, of how aging induces epigenetic/miRNA changes that contribute to the deterioration of collateral function that accompanies the aging process.

Disclosures

None.

References


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Stephen E. Epstein, Roberta M. Lassance-Soares, James E. Faber and Mary Susan Burnett

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