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Proinflammatory Arterial Stiffness Syndrome: A Signature of Large Arterial Aging

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Aging · Chronic stress · Proinflammation · Arterial remodeling · Arterial stiffening

Abstract
Age-associated structural and functional remodeling of the arterial wall produces a productive environment for the initiation and progression of hypertension and atherosclerosis. Chronic aging stress induces low-grade proinflammatory signaling and causes cellular proinflammation in arterial walls, which triggers the structural phenotypic shifts characterized by endothelial dysfunction, diffuse intimal-medial thickening, and arterial stiffening. Microscopically, aged arteries exhibit an increase in arterial cell senescence, proliferation, invasion, matrix deposition, elastin fragmentation, calcification, and amyloidosis. These characteristic cellular and matrix alterations not only develop with aging but can also be induced in young animals under experimental proinflammatory stimulation. Interestingly, these changes can also be attenuated in old animals by reducing low-grade inflammatory signaling. Thus, mitigating age-associated proinflammation and arterial phenotype shifts is a potential approach to retard arterial aging and prevent the epidemic of hypertension and atherosclerosis in the elderly.

Introduction

Aging is a major risk factor for the morbidity and mortality caused by cardiovascular disease. Systemic aging is defined as an age-related decline in physiological function primarily driven by chronic exposure to low levels of sterile inflammation, known as "proinflammation," contributing to cellular senescence and pathological aging [1, 2]. Arterial aging is the cornerstone of systemic aging and is mainly driven by local proinflammation [3–5]. Age-associated proinflammatory cellular and matrix modifications are the foundation for an exponential increase in the pathogenesis of hypertension and atherosclerosis [3, 6].

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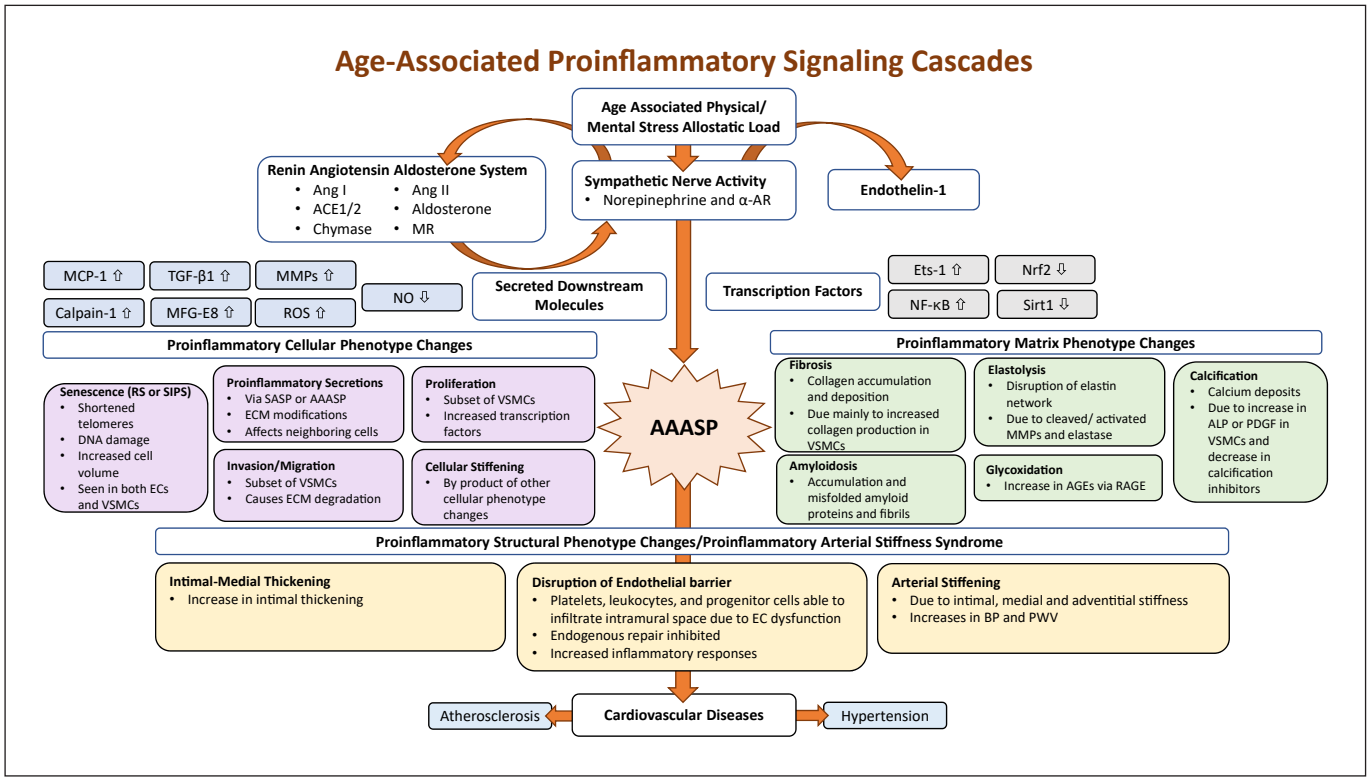

Fig. 1. Age-associated proinflammatory signaling cascades at the molecular, cellular, and tissue levels in the arterial wall. The age-associated vascular molecular cascades are triggered by changes due to physical/mental stress allostatic load on the organism. These molecular changes impact the interconnected RAAS and the SNA systems, thus, activating ET-1, and lead to the secretion of downstream molecules and transcription factors. α-AR, α-adrenergic receptor; ACE, angiotensin-converting enzyme; AAASP, age-associated arterial secretory phenotype; AGEs, advanced glycation end products; ALP, alkaline phosphatase; Ang, angiotensin; BP, blood pressure; EC, endothelial cell; ECM, extracellular matrix; Ets-1, the v-ets erythroblastosis virus E26 oncogene homolog 1; MCP-1, monocyte chemoattractant protein-1; MFG-E8, milk fat globule epidermal growth factor-8; MMPs, matrix metalloproteases; MR, mineralocorticoid receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, NF-E2-related factor 2; NO, nitric oxide; PDGF, platelet-derived growth factor; PWV, pulse wave velocity; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; RS, replicative senescence; SASP, senescence-associated secretory phenotype; Sirt1, silent information regulation 2 homolog 1; SIPS, stress-induced premature senescence; TGF-β_1, transforming growth factor-β_1; VSMC, vascular smooth muscle cell.

Age-associated arterial proinflammation is mainly generated by vascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) [3]. Cellular proinflammation is tightly regulated by sympathetic nerve activity (SNA), the renin angiotensin aldosterone system (RAAS), and endothelin activities induced by physical and mental stress known as allostatic load [7–16]. This chronic stress is the cost that the organism pays for trying to maintain stability in a changing environment over a lifetime.

A comprehensive view of arterial aging is illustrated in Figure 1. At the molecular level, proinflammatory cytokines and chemokines accumulate within the arterial wall. At the cellular level, vascular cells shift phenotypically to heterogeneous phenotypes: a subset of VSMCs becomes senescent, while another subset of cells becomes more proliferative, invasive/migratory, secretory, and stiff. The extracellular matrix demonstrates fibrosis, elastolysis, calcification, amyloidosis, and glycoxidation. Finally, at the tissue level, proinflammation increases arterial intimal-medial thickening (IMT), endothelial dysfunction, arterial stiffening, and elevated blood pressure (BP). These tissue-level changes comprise "proinflammatory arterial stiffness syndrome," a clinical change that does not necessarily evolve into cardiovascular disease. Thus, inhibiting age-associated proinflammation may be a novel approach for maintaining a healthy vasculature and curbing the epidemic of cardiovascular disease in the aging population.

Molecular Phenotypes in the Aging Arterial Wall

Age-Associated Leading Stressors

We recognize that increases in the key molecules of SNA, the RAAS, and endothelin-1 (ET-1) activity are the leading proinflammatory stressors in arterial aging based upon recently published studies (Fig. 1).

Norepinephrine

Aging increases SNA, which is characterized by an increase in neurohormone norepinephrine secretion in the arterial wall and an upregulation of its receptor, α-adrenergic receptor [9, 10, 17, 18]. SNA is also interconnected with the RAAS and ET-1. Increased SNA contributes to endothelial dysfunction, vasoconstriction, IMT, and BP increase and increases arterial proinflammation [8, 9, 14, 19].

Angiotensin II

Increased SNA triggers activation of the RAAS at both central and peripheral levels with aging [9, 12, 14, 19]. The transcription, translation, and activity of angiotensin-converting enzyme (ACE) markedly increases in the arterial wall with aging [20–26]. Chymase, an alternative angiotensin convertase, also increases in expression within the arterial adventitia [22]. Both ACE and chymase cleave angiotensin I (Ang I) into angiotensin II (Ang II). Consequently, Ang II protein is significantly elevated in the aging arterial wall [21–23, 25, 27–29], along with increased expression of the Ang II receptor, AT1 [21, 30]. The "aging-elevated" Ang II/AT1 expression in the arterial wall signals to the SNA, subsequently creating an inflammatory environment which contributes to arterial remodeling.

Aldosterone

Aldosterone (Aldo), a component of the RAAS and a downstream Ang II effector, is also regulated by SNA, and is known as the sympathetic-adrenal system [9]. Aldo, secreted by the adrenal glands, binds to the mineralocorticoid receptors (MRs). Aging increases the clustered zona glomerulosa cells of the adrenal glands and subsequently enhances the production and secretion of Aldo, called "age-related autonomous aldosteronism" [31, 32]. Further, aging also increases the local expression of MR in arterial walls and cells [33–35]. Elevated Aldo/MR signaling enhances extracellular signal-regulated kinase (ERK) 1/2 signaling, contributing to the proinflammatory phenotypic shift of VSMCs, thus promoting vasoconstriction, stiffening, and BP increase [33–37].

Endothelin-1

The endothelium, known as the body's largest "endocrine gland," produces ET-1, and is also regulated by SNA [14, 38]. With advancing age, aortic proendothelin-1 (pro-ET-1) and activated matrix metalloproteinase (MMP) type II (MMP-2) levels increase [39]. Pro-ET-1 can be cleaved into an active ET-1 peptide by either endothelin-converting enzyme or MMP-2 within the arterial walls [39–41]. Consequently, active ET-1 levels are increased in aging [38, 39, 42]. ET-1 enhances inflammation by increasing the expression of the transcription factor E26 transformation-specific proto-oncogene 1 (ets-1) in VSMCs with aging [39]. Aging increases the sensitivity of the ET receptor in aortic walls, further augmenting proinflammation [43].

Age-Associated Key Secreted Downstream Molecules

The interrelationship of the SNA/RAAS/ET-1 signaling pathways promotes a proinflammatory response in aged arterial walls, leading to an increase in key downstream molecules (Fig. 1). Aged cells, including senescent cells, modify their microenvironment via the secretion of a variety of bioactive factors known as the age-associated arterial secretory phenotype (AAASP), including the senescence-associated secretory phenotype (SASP) [3].

Monocyte Chemoattractant Protein-1

Monocyte chemoattractant protein-1 (MCP-1) increases in aged arterial walls in association with both SNA and Ang II signaling [44, 45]. ET-1 also promotes the secretion of MCP-1 in aged VSMCs [39]. Both mRNA and protein levels of MCP-1 are upregulated within the aged aortic wall [45–47]. Increases in MCP-1 protein expression are mainly localized to the thickened intima and promote VSMC proinflammation [45–47]. MCP-1 is a key intermediary signaling molecule which connects the SNA/RAAS/ET-1 signaling pathway to proinflammatory cellular and matrix phenotypical changes.

Transforming Growth Factor-β_1

Transforming growth factor-β_1 (TGF-β1)/TGF beta receptor type II activation is a powerful fibrotic signaling cascade that is closely mediated by Ang II/AT1 signaling [48–51]. The levels of secreted TGF-β_1 protein from VSMCs are upregulated in aged rat aortae [49]. Increased collagen synthesis, secretion, and deposition are triggered by interactions between TGF-β_1/TGF beta receptor type II and p-SMAD-2/3 signaling [39, 48, 49, 52]. In addition, treating ECs with TGF-β_1 peptide also increases the expression of collagen types I and III [53, 54]. Interestingly, MCP-1 has

been shown to colocalize with TGF-β_1 within the arterial wall to enhance the activity of TGF-β_1 in VSMCs [55]. Thus, interactions of TGF-β_1 with MCP-1 may play a significant role in age-associated arterial fibrosis.

Matrix Metalloproteinases

MMPs degrade the extracellular matrix. MMP-2 is a downstream molecule of both Ang II and phenylephrine signaling in the arterial wall and cultured VSMCs [27]. Both the mRNA and protein levels of MMP-2/9 are upregulated in aged aortic walls [22, 27, 49, 55–57]. The increased ratio of the MMP activator membrane-type 1 matrix metalloproteinase to the MMP tissue inhibitor of MMP-2 potentially promotes MMP-2/9 activation with aging [21, 22, 58]. Activated MMP-2/9 is predominantly located at the thickened intima and the inner media of arteries [22, 58]. Notably, secreted activated MMP-2/9 from VSMCs is upregulated with aging, which mainly contributes to an increased activation of arterial MMP-2/9. Importantly, activated MMP-2 increases the bioavailability of proinflammatory vasoactive molecules, e.g., cleaves latent transforming protein-1 and pro-ET-1 into activated TGF-β_1 and ET-1 in the arterial wall or VSMCs [39–41, 49].

Calpain-1

Calpain-1 is a calcium-dependent intracellular protease which modulates extracellular MMP-2 and TGF-β_1 activity in the aged arterial wall or cultured VSMCs [29, 59]. The activity of calpain-1 is significantly increased in both aged rat aortae and cultured aortic VSMCs [29] and facilitates activation of MMP-2 and TGF-β_1, leading to fibrosis and calcification [59]. In contrast, the calpain-1 inhibitor BDA-1 attenuates aortic calcification in aging klotho-deficient mice [60]. Ang II both activates and colocalizes with calpain-1 in the aged arterial wall and VSMCs [29]. Thus, calpain partially relays the proinflammatory signaling of Ang II in the aged arterial wall or cultured VSMCs.

Milk Fat Globule-EGF-8

Milk fat globule-EGF-8 (MFG-E8) is a highly glycosylated protein enriched in milk fat globule-containing EGF and blood-clotting factor VIII. Aging not only increases MFG-E8 mRNA and protein levels but also increases its fragment medin in the arterial wall [61–63]. Treating aged VSMCs with MFG-E8 increases the proliferation and migration of VSMCs. Medin has a strong affinity to elastin fibers, promoting elastolysis and amyloidosis in the arterial wall [62–65]. In addition, Ang II induces MFG-E8 protein expression, which increases MCP-1 expression in VSMCs, promoting proinflammation [28]. Notably, MFG-E8 is also a well-known bridging molecule that mediates the clearance of apoptotic cells (efferocytosis) by macrophages, retarding the growth and vulnerability of atherosclerotic plaques in aging mice [66–68]. The complex role of MFG-E8 in the aged proinflamed arterial wall needs to be further explored.

Reactive Oxygen Species

Reactive oxygen species (ROS) are increased in the aged arterial wall or VSMCs. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression is the main source of production of arterial ROS. Further, levels of the antioxidant proteins Cu/Zn SOD (SOD1), Mg SOD (SOD2), and extracellular matrix superoxide dismutase (ECM-SOD/SOD3) are downregulated during aging [69–72]. Therefore, aging creates an imbalance of NADPH oxidase and dismutase in the arterial wall, eventually augmenting the increase in ROS levels. This imbalance, along with increases in both Ang II and ET-1, enhances NADPH expression and the production of ROS [14, 25, 52, 69, 73–77]. Increased ROS modifies proinflammation, endothelial dysfunction, and arterial stiffening in the arterial wall with aging [25, 52, 72, 78–82].

Nitric Oxide and Bioavailability

Nitric oxide (NO), a small diffused signaling molecule, regulates arterial dilatation, stiffening, and inflammation with aging [25, 69, 72, 74, 83–87]. Endothelial NO synthase activation determines the production of NO in the arterial wall. Expression of arterial endothelial NO synthase is decreased and contributes to a reduction in NO production in the aged arterial wall [70, 84, 88–90]. In addition, NO interacts with ROS to generate peroxynitrite ($ONOO^-$). This ROS further decreases the bioavailability of NO, impairs endothelium-dependent relaxation, and enhances vasoconstriction and proinflammation [74, 90, 91].

Age-Associated Transcription Factors

RAAS/SNA/ET-1 signaling contributes to the activation or inactivation of nuclear transcription factors that are key intermediary molecules contributing to proinflammatory cellular and matrix phenotypical changes (Fig. 1).

Ets-1 and NF-κB

The major proinflammatory transcription factors Ets-1 and NF-κB are activated in the aged arterial wall. Increased Ets-1 activity is closely associated with increased

transcription levels of ET-1, MCP-1, TGF-β_1, and MMP-2 [39]. Increased NF-κB activity in old arterial cells promotes oxidative stress and triggers an inflammatory response [92–94].

Nrf2 and SIRT1

The major anti-proinflammatory transcription factors nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and sirtuin (silent mating type information regulation 2 homolog) 1 (*S. cerevisiae*) (SIRT1) are decreased in the aged arterial wall. Nrf2 protects against oxidative stress and its related cytotoxic effects and magnifies NF-κB activation [95]. SIRT1 is downregulated and inactivated with aging [71, 83, 87, 96, 97]. Decreased Sirt1 activity increases NADPH oxidase-dependent ROS production, senescence, and inflammation and enhances endothelial dysfunction in the aged arterial wall [83, 96–98].

Summary

With advancing age, both physical and mental stress increases due to continuous adaptations to changes in the living environment. Increased stress triggers the activation of both the RAAS and SNA, leading to ET-1 activation. These "leading proinflammatory signaling" events act on arterial cells by directly promoting the secretion or production of MCP-1, TGF-β_1, MMPs, calpain-1, and MFG-E8, known as the AAASP, as well as the activation or inactivation of transcription factors Ets-1, NF-κB, Nrf2, or Sirt1. These age-associated proinflammatory molecular phenotype alterations eventually lead to age-associated cellular and matrix phenotypical changes. Further studies are needed to elucidate the finer details of the mutable events that facilitate the directionality and movement of the proinflammatory signaling cascade with aging as illustrated in Figure 1.

Cellular and Matrix Phenotypes in the Aging Arterial Wall

The structure and function of the arterial cells and matrix are remodeled with advancing age through proinflammatory signaling. Changes in age-associated cellular and extracellular phenotypes are illustrated in Figure 1.

Age-Associated Cellular Phenotypes

EC Senescence

The number of arterial ECs decreases with aging potentially due to either replicative senescence (RS) with telomere reduction and inactivation of telomerase, or stress-induced premature senescence (SIPS) without telomere involvement. Indeed, the number of both RS and SIPS ECs increases in the aged arterial wall [81, 85, 88, 99–102]. A subset of aged ECs appears with decreased mitotic frequency, an increase in cellular volume, and shortened telomeres while entering an RS state [103–105]. In addition to RS, the Ang II signaling cascade plays a significant role in the SIPS of ECs via a reduction of Sirt1 and ERK1/2/BCL2 signaling, and functional autophagy in addition to increased ROS production [81, 100–102].

VSMC Senescence

A subset of aged VSMCs appears enlarged and becomes senescent in the arterial wall [97, 98, 100, 106–110]. Oxidative stress and DNA damage cause VSMC senescence and are linked to shortened telomeres or SIPS triggered through Ang II signaling [82, 100, 111, 112]. Increased senescence is associated with an increase in p16 expression, a loss of SIRT1 expression, and an upregulation of miR-34a in VSMCs. In addition, the mutant lamin A, known as progerin, also drives the senescence of VSMCs [110, 113].

Proliferation

VSMC proliferation is increased in the aged arterial wall. Age-associated secretory molecules, such as MFG-E8, may promote a replicative subset of VSMCs [108, 114]. This subset of VSMCs has an increased proliferative capacity via MFG-E8 signaling, displaying increased ERK1/2 phosphorylation and 5-bromo-2′-deoxyuridine incorporation, as well as increased expression of proliferating cellular nuclear antigen, cyclin-dependent kinase 4, and platelet-derived growth factor (PDGF-BB) [115].

Invasion/Migration

VSMC invasion is the ability to migrate or infiltrate neighboring tissue through the vascular extracellular matrix. Interestingly, the invasive capacity of a VSMC subset is increased in the aged arterial wall, contributing to diffuse intimal thickening [3, 108, 114, 116]. Aging increases the signaling of Ang II, which triggers the secretion of calpain-1, MFG-E8, or MCP-1 in VSMCs, and also activates MMP-2 [27–29]. Increased MMP-2 activation is the key molecule that drives the invasive capabilities of VSMCs via a breakdown of their basement membrane and surrounding extracellular matrix [21, 28, 29, 55]. Conversely, the invasive capacity of old VSMCs can be inhibited by the MMP-2 inhibitor GM6001 [46, 55].

Stiffening

Stiffening of VSMCs is a central and mutable element in arterial aging [78, 117–121]. VSMCs derived from older animals demonstrate increased stiffness compared to similar cells derived from young adults [119, 122]. VSMC stiffness is highly sensitive to microenvironmental molecules such as TGF-β_1 and transglutaminase 2 [119, 123]. TGF-β_1 serves as a specific modifier of age-associated VSMC stiffening through the clustering of mechanosensitive $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins [122]. Increased stiffness in aging is also dependent on the expression and organization of the VSMC cytoskeletal proteins along the arterial tree [123, 124]. Interestingly, a stiffened substrate reinforces VSMC stiffening [125]. The stiffness correlates with phenotypic changes of VSMCs [123]. Increased stiffening is converted into proinflammatory signaling in the aged arterial wall [126].

Proinflammatory Secretions

Aged vascular cells, including senescent cells, modify their microenvironment via the autonomous or nonautonomous secretion of a variety of bioactive factors. The vascular SASP includes cells that promote proinflammation of neighboring cells [108, 114]. Interestingly, growing evidence indicates that aged primary isolated VSMCs from aortic walls have a unique chemokine and cytokine proinflammatory profile (the AAASP), which also drives proinflammation in neighboring cells [5, 92]. MMP-2, MCP-1, TGF-β_1, MFG-E8, and TNF-α are characteristic proteins secreted from old VSMCs with the AAASP [3, 92].

Age-Associated Matrix Phenotypical Changes

The extracellular matrix of arterial walls is modified by age-associated proinflammatory secretions of vascular cells through fibrosis, elastolysis, calcification, and amyloidosis [47, 85, 97, 99, 127].

Fibrosis

Fibrosis develops through an increase in collagen deposition in the arterial walls of aging rats [58, 75]. Collagen accumulation also significantly increases within the arterial walls of aged humans [128]. Secreted MMP-2 activates TGF-β_1 and promotes VSMC collagen production [108, 114]. Collagen deposition within the interlamellar layers of the arterial wall plays a significant mechanical role in arterial stiffening [129].

Elastolysis

An intact interlamellar elastin layer is important for the health of large arteries. The aging interlamellar elastin network is disrupted and collapsed in elastolysis due to cleavage by MMPs and elastase [58, 130–132]. Elastolysis is observed with an increase in the amounts of activated MMP-2/9 or elastase in the interlamellar elastin network [50, 55]. This age-associated destruction of the interlamellar elastin lamina is also associated with loss of tropoelastin production, impairing rejuvenation [133]. The destruction of vascular interlamellar elastin results in an eventual decrease in arterial elastic energy storage capability, compliance, and resilience [134]. In addition, short peptides, released during elastolysis, known as "elastokines," actively participate in the onset and progression of arterial inflammation and calcification.

Calcification

Arterial calcification plays a crucial role in the development of arterial stiffening [135]. Increased calcium deposits, an element of calcification, are markedly increased in the arterial wall with aging [136, 137]. The morphology of older VSMCs appears osteoblast-like, producing large amounts of bone-like substrates such as collagen II [59]. The development of arterial calcification is dependent upon a balance of pro- and anti-calcification molecules. Overexpression of alkaline phosphatase, a pro-calcification molecule, increases arterial calcification and is one of the pro-calcification molecules that is found with greater frequency in old or senescent VSMCs. In addition, anti-calcification molecules, such as osteonectin and osteopontin, are simultaneously reduced in old VSMCs [59]. Notably, age-associated increases in PDGF, a powerful cellular mitogen, also significantly accelerate the process of arterial calcification [138].

Amyloidosis

With advancing age, misfolded aggregated amyloid proteins and fibrils are increased in arterial walls [62–65]. One of the main constituents of arterial amyloid fibrils is a 5.5-kDa fragment of MFG-E8, known as medin, which is markedly increased in the aged arterial wall [61, 62, 65]. Medin has a high capacity for binding to elastin fibers, potentially increasing stiffness and calcification [61–65]. Thus, medin amyloid is implicated as greatly affecting the elasticity of aged arteries and therefore needs further investigation.

Advanced Glycation End Products

Advanced glycation end products (AGEs) are increased in the aged arterial wall. It is well known that AGE accumulation contributes to multiple structural and functional alterations in the arterial system, such as

senescence, proinflammation, and stiffening [4, 52, 139–141]. AGEs are often generated by reactions between sugar chains and biologic amines of oxidized collagen. Older, cleaved/degraded, oxidized collagen fibers are common molecular targets that are easily modified via a reaction between ROS and sugars in the arterial wall. Aging increases AGEs and promotes collagen production through activation of its receptor, RAGE, in a feedback manner.

Summary

Aging can be described as a form of subclinical pathological conditions. Proinflammatory molecular signaling acts on arterial cells, generating age-associated cellular and matrix phenotypical changes, as illustrated in Figure 1. These phenotypes are all observed in the aged arterial wall and have been reproduced in vivo and in vitro. These characteristic cellular and matrix alterations can also be induced in young animals under experimental proinflammatory stimulation. Clinical proinflammatory structural phenotypical changes may be observed in aged populations without evidence of cardiovascular disease. The signaling by these phenotypes is complex, and it is unknown how multiple cellular and matrix events are controlled and how a subclinical symptom evolves into a clinical disease. For example, how does the arterial stiffness syndrome evolve into a clinical pathological condition such as hypertension and atherosclerosis? It is important to decode the signaling network and find the key switch for the diagnosis, prevention, and treatment of adverse arterial remodeling with aging.

Arterial Subclinical Phenotypes with Aging

Age-associated cellular and extracellular phenotypic shifts ultimately lead to "arterial proinflammatory stiffness syndrome," including IMT, endothelial dysfunction, stiffening, and BP increase (Fig. 2).

Intimal-Medial Thickening

IMT can be accurately evaluated by B-mode ultrasound and other noninvasive imaging techniques and is a hallmark of age-associated arterial remodeling [3, 8, 23, 116, 142–149]. Expansion of the intimal layer, rather than the media, is mainly responsible for increased IMT [150], which is linked to increases in both vascular relaxation and stiffening [143, 144, 151, 152]. Notably, there is also an increase in VSMC progenitor cells promoting age-associated IMT [153].

Endothelial Dysfunction

The arterial endothelial barrier becomes disrupted with advancing age. More senescent ECs, with reduced telomerase, contribute to endothelium-dependent dysfunction by increasing the number of defective sites along the lumen [85, 88, 99, 154, 155]. Circulating platelets adhere and infiltrate via the damaged sites into the arterial wall, initiating inflammatory responses. Increased adhesive platelets on the inner surface of the arterial lumen not only inhibit the proliferation and migration of local ECs but also exhaust endogenous repair by progenitor cells [99]. Therefore, increases in activated and aggregated platelets damage the integrity of the aged arterial endothelial barrier and also promote endothelial dysfunction [145]. Furthermore, EC contractility is enhanced with aging, leading to increased endothelial permeability and intimal stiffening [25, 26, 38, 72, 83, 85, 87, 93, 148].

Aging also increases monocytosis and enhances macrophage transdifferentiation and accumulation within the aorta [145, 156]. The accumulation of macrophages within the arterial wall leads to metabolic impairment and subsequently accelerates arterial remodeling [145, 157]. Increased amounts of activated neutrophils and lymphocytes infiltrate the intramural space and interact with ECs and VSMCs, facilitating ROS production, senescence, and endothelial function [68, 145, 158, 159]. In addition, older subjects with greater cardiovascular risk factors have lower numbers of circulating endothelial progenitor cells, which is linked to the endogenous regenerative potential, suggesting the reparative capacity of the endothelium is decreased [142].

Arterial Stiffening

Arterial stiffness, including intimal, medial, and adventitial stiffness, is dependent on an intrinsic stress/strain relationship determined by both cell and matrix stiffness [77, 118, 120, 123, 125, 135, 139, 147, 148, 151, 154, 160–163]. Pulse wave velocity (PWV) has emerged as a gold standard for the noninvasive assessment of central arterial stiffness, a predictor of the incidence of hypertension and all-cause mortality, and increases with advancing age [144, 151, 162–164]. The Baltimore Longitudinal Study of Aging (BLSA) demonstrated that increased PWV was associated with increased systolic BP and also a greater incidence of hypertension [165]. Further, the aortic-brachial PWV ratio has emerged as a novel index of BP independent of vascular aging [144], and the carotid-radial/carotid-femoral PWV ratio is an accurate predictor of all-cause mortality [151].

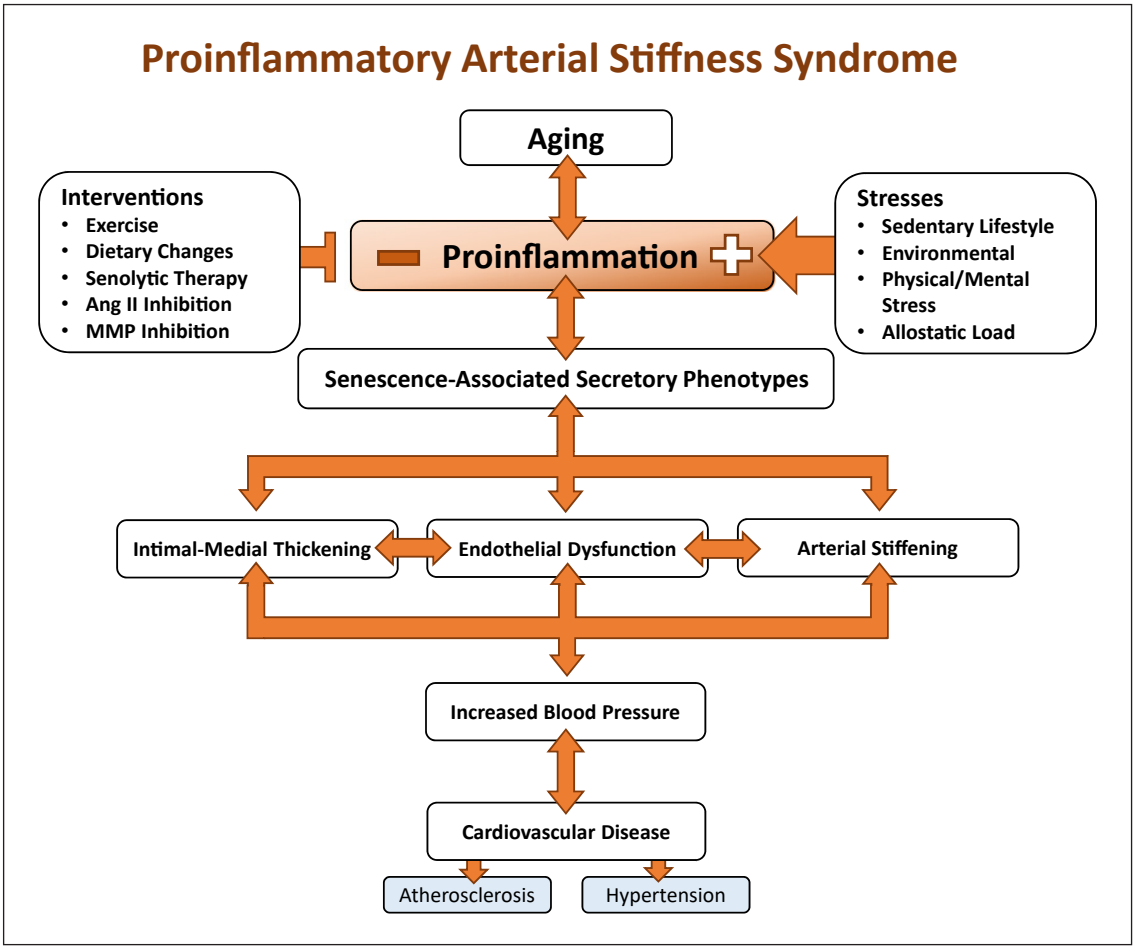

Fig. 2. Diagram of proinflammatory arterial stiffness syndrome. The phenotype changes made at the cellular and matrix level characterize the final stage of the vascular aging cascade that ultimately leads to proinflammatory structural phenotype changes and eventually cardiovascular disease. Ang II, angiotensin II; MMP, matrix metalloprotease.

BP Increase

Increases in systolic BP and pulse pressure occur after the sixth decade of life, becoming a hallmark of arterial aging [135, 143–145, 147, 160, 166]. Further, BP measurements are closely associated with PWV [143].

Summary

The accumulation of the above-mentioned molecular, cellular, and matrix phenotypes in the arterial wall with aging manifests as arterial proinflammatory stiffness syndrome. The clinical arterial phenotype is illustrated in Figure 2, including IMT, endothelial dysfunction, stiffening, and BP increase. These subclinical conditions are detected in the elderly without overt cardiovascular events, which we regard as arterial proinflammatory syndrome. Further studies are needed to understand the mechanism by which accelerated aging leads to the clinical arterial phenotype and reduces the disease threshold which may ultimately lead to clinical cardiovascular disease.

Interventions to Counteract Arterial Aging

Since proinflammation is central to arterial aging, efforts to reduce proinflammation could help reduce the clinical progression of arterial aging. A healthy lifestyle and regular exercise can prevent age-associated senescence and secretion.

Pharmacological interventions may act to disrupt the progression of vascular aging by inhibiting the AAASP/

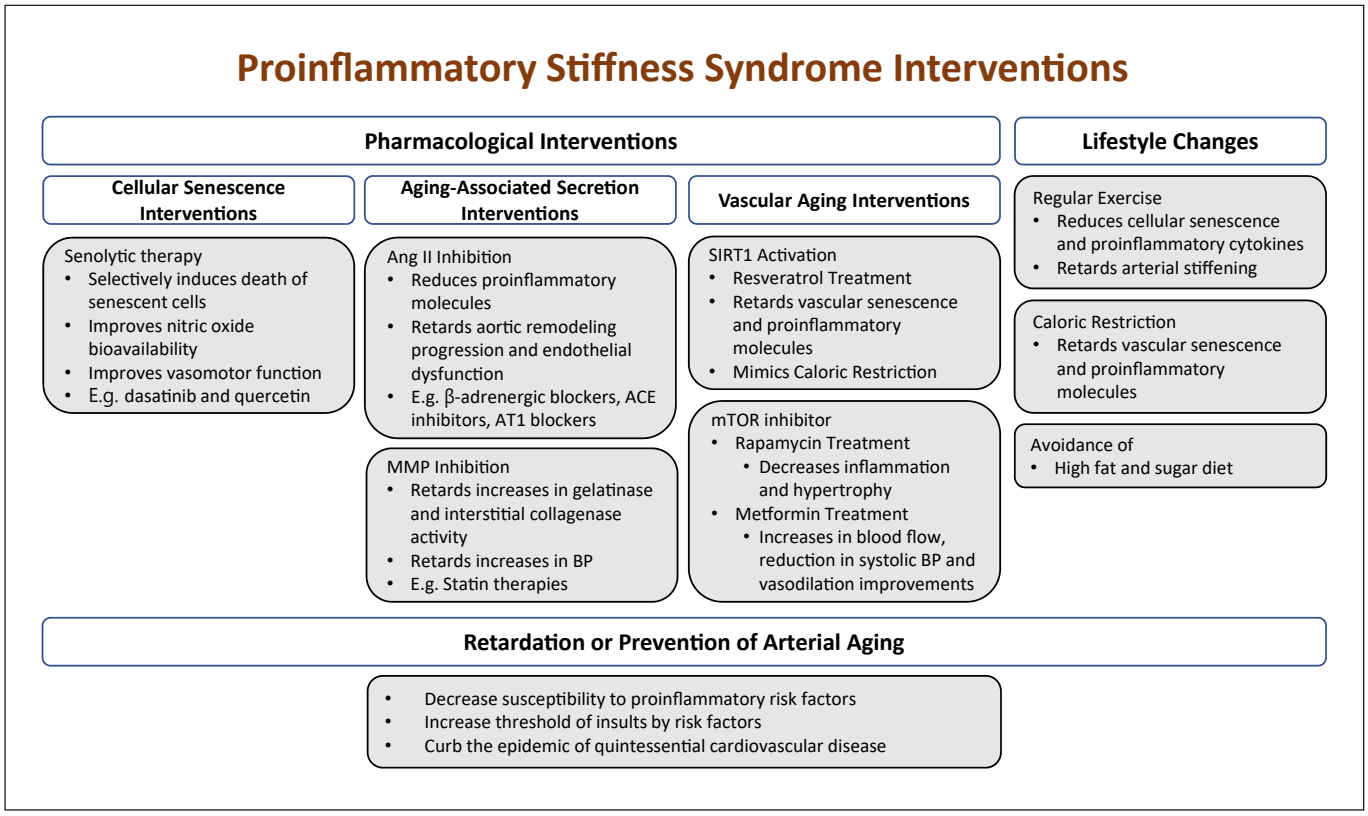

Fig. 3. Age-associated proinflammatory arterial stiffness syndrome interventions. Vascular aging can be mitigated through various approaches that target the proinflammatory cascades. The eventual structural phenotypical changes are not inevitable, and cardiovascular disease may be prevented. ACE, angiotensin-converting enzyme; Ang II, angiotensin II; AT1, Ang II receptor; BP, blood pressure; MMP, matrix metalloprotease; mTOR, mammalian target of rapamycin; SIRT1, silent information regulation 2 homolog 1.

SASP directly or, through the removal of senescent vascular cells with senolytic drugs, may modify arterial aging and diminish the proinflammatory cascades [2, 167–169] (Fig. 3).

Diet

Long-term caloric restriction retards both vascular senescence and proinflammatory molecules by decreasing MCP-1, ROS, and MMP activation and improving NO bioavailability and endothelial function, thereby preventing arterial stiffening with aging [86, 170–172]. Not only are these molecules disrupted, but the clinical signs of aging – such as increased body weight, BP, cholesterol levels, and arterial stiffness – improve [86, 170–172].

A high-fat-and-sugar (HFS) diet drives arterial aging in nonhuman primates [170, 171]. Animals fed on an HFS diet for 2 years not only showed increases in body weight and circulating cholesterol, but also exhibited signs of central arterial wall stiffening and inflammation [164]. Further study showed that the stiffening-associated loss of EC integrity, lipid and macrophage infiltration, and calcification of the arterial wall were driven by genomic and proteomic disorders of oxidative stress and inflammation [164].

Exercise

Regular exercise substantially reduces ET-1 signaling, TGF-β_1 activity, cellular senescence, proinflammation, and calcification in the aged arterial wall [76, 78, 86, 89, 141, 173]. Importantly, regular exercise effectively prevents age-associated SNA, BP increase, arterial stiffening, and endothelial dysfunction [11, 16, 78, 89, 152, 174].

Senolysis

Clearance of senescent cells using transgenic and pharmacological approaches retards arterial aging. Senolytics, such as dasatinib and quercetin, have been utilized to retard vascular aging. Dasatinib primarily eliminates senescent progenitor cells, while quercetin is more effective

against senescent ECs [175]. Combined treatment with dasatinib and quercetin significantly reduces the arterial senescent cell burden in the arterial wall, increases NO bioavailability, and improves vasomotor dysfunction with aging [85]. Senolytic therapy can also reduce the progress of age-associated atherosclerosis [176, 177].

Inhibition of Ang II Signaling

The Ang II proinflammatory signaling cascade has been widely studied [20, 24–26, 44, 81, 178]. Chronic administration of Ang II to young rats not only increases their BP, but also enhances the activity of MMP-2, TGF-β_1, calpain-1, and MFG-E8 as well as collagen production within the arterial wall, similar to that in untreated old animals [27, 29, 59, 143, 179]. In contrast, chronic ACE inhibition of the Ang II receptor, an AT_1 antagonist, significantly reduces the abundance of proinflammatory molecules and retards the progression of adverse aortic remodeling in experimental aging animals [20, 24–26, 178].

Inhibition of MMPs

Activated MMPs are common elements of the SASP and AAASP. Chronic administration of PD166793, a broad-spectrum MMP inhibitor, significantly retards age-associated increases in gelatinase and interstitial collagenase activity, ET-1 expression, elastic fiber fragmentation, and collagen production in the arterial wall of rats [39]. Interestingly, MMP inhibition also substantially retards increases in BP with advancing age [39].

Activation of SIRT1

Resveratrol treatment, a caloric restriction-mimicking small molecule and an agonist of SIRT1, effectively prevented HFS diet-induced arterial wall inflammation and arterial stiffening in nonhuman primates [164]. These findings suggest that dietary resveratrol, like caloric restriction, promises to ameliorate age-associated arterial inflammation, elastolysis, and stiffening [81, 92, 96, 180].

Inhibition of mTOR

Other treatments, like rapamycin and metformin therapy, focus on decreasing inflammation and arterial stiffening and improving endothelial dysfunction through the inhibition of mTOR (mammalian target of rapamycin) [181, 182].

Summary

Pharmacological and lifestyle interventions against age-associated proinflammatory stiffness syndrome are illustrated in Figure 3. These interventions are largely derived from studies of experimental animals, and it is difficult to translate these approaches to the clinic. It is important to perform a large double-blind, random clinical trial to find the most effective time, dose, and side effects of potential treatments. Since advanced aging is a form of subclinical disease, targeting proinflammation may be the best approach to mitigating cardiovascular disease, which evolves into clinical conditions through either reduced disease thresholds or increased susceptibility and vulnerability.

Concluding Remarks

Age-associated arterial structural and functional remodeling are driven by chronic increases in proinflammatory signaling causing proinflammatory stiffness syndrome. At the cellular level, increases in senescent and senescence-associated molecular signaling have been observed both in vivo and in vitro. Microscopically, IMT, endothelial disruption, cellular senescence, and senescence-associated cellular and matrix phenotypes are characteristics of the aged arterial wall. These adverse molecular, cellular, and matrix events, presenting as "old age phenotypes," are also observed in young animals experimentally infused with proinflammatory stimulants. Alternatively, in old animals, these adverse remodeling events are alleviated by inhibition of cellular senescence and senescence-associated phenotypes, resulting in more "youthful phenotypes."

Arterial senescence and senescence-associated heterogeneous phenotypes cause proinflammatory stiffness syndrome, presenting as a subclinical condition, and they may prepare the ground for the initiation and progression of hypertension and atherosclerosis at the molecular, cellular, and vascular levels. Thus, interventions that suppress or prevent proinflammatory stiffness syndrome at different levels may hold great promise in treating and preventing age-associated vascular diseases such as hypertension and atherosclerosis. Questions still remain regarding the mutability of the proinflammatory cascades and the triggers that control each level. Future studies are needed to decode the proinflammatory signaling network and understand how subclinical conditions evolve into cardiovascular diseases.

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