



Angiotensin-(1–7), a protective peptide against vascular aging

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ABSTRACT

Vascular aging is a complex and multifaceted process that provokes profound molecular, structural, and functional changes in the vasculature. Eventually, these profound aging alterations make arteries more prone to vascular disease, including hypertension, atherosclerosis and other arterial complications that impact the organism beyond the cardiovascular system and accelerate frailty. For these reasons, preventing or delaying the hallmarks of vascular aging is nowadays a major health goal, especially in our aged societies. In this context, angiotensin(Ang)-(1–7), a major player of the protective branch of the renin-angiotensin system, has gained relevance over recent years as growing knowledge on its anti-aging properties is being unveiled. Here, we briefly review the main actions of Ang-(1–7) against vascular aging. These include protection against vascular cell senescence, anti-inflammatory and antioxidant effects together with the induction of cytoprotective systems. Ang-(1–7) further ameliorates endothelial dysfunction, a hallmark of vascular aging and disease, attenuates fibrosis and calcification and promotes protective angiogenesis and repair. Although further research is needed to better understand the anti-aging properties of Ang-(1–7) on the vasculature, this heptapeptide arises as a promising pharmacological tool for preventing vascular aging and frailty.

1. Vascular aging and cardiovascular disease

The concept that a person is as old as his/her arteries are, reformulated by Sir William Osler more than 100 years ago, is still valid nowadays. Vascular aging is a most influencing cause affecting the health in the elderly, and, in fact, aging is the main risk factor for the development of cardiovascular (CV) diseases.

The aging process is associated with complex structural, molecular and functional changes in the vasculature, even in the absence of other CV risk factors, such as hypertension, obesity, diabetes, or hypercholesterolemia. Eventually, these profound aging alterations make arteries more prone to hypertension, atherosclerosis, medial degeneration and the onset of a different array of arterial complications (i.e. myocardial infarction, stroke, aneurysms) [1,2].

Although traditionally considered inexorable, many researchers currently highlight the need for understanding the cellular and molecular mechanisms involved in age-related vascular dysfunction. Improving our knowledge will allow for developing strategies to

attenuate vascular aging, and thus for preserving the quality of life and alleviating CV diseases in the elderly or in pathological states, such as type 2 diabetes mellitus and obesity, exhibiting premature vascular aging [3]. Furthermore, preservation of vascular function in aging should not only reduce deaths and disabilities secondary to CV events but should also influence other aspects of the aging process that lead to loss of function and/or disability, such as motor capacity and cognitive frailty [4]. Indeed, it is now widely accepted that robustness cannot be achieved without the preservation of vascular function.

2. The hallmarks of vascular aging

As stated above, vascular aging is a multifaceted process that implies profound functional and structural changes. These changes affect vascular cells, mostly endothelial and vascular smooth muscle cells (VSMC), and alter the communication routes and the cross-talk between these two cell types [2]. Other non-cellular components of the vessel wall, like the extracellular matrix, are also deeply affected by the aging

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process [2].

Globally, aged vessels display a wide array of features including intima-media thickness, calcification, vascular stiffness and matrix metalloproteinase dysregulation, endothelial dysfunction, increased expression of inflammatory molecules, altered inter-cellular communication, aggravated oxidative stress, vascular cell senescence, or shortened telomere length, among other [5,6].

Many different factors have been identified to date as culprits of accelerating vascular aging. These include the over-activation of the renin-angiotensin system (RAS) or the excess generation of pro-inflammatory cytokines and related pathways, among other. Less literature is nevertheless available on those players that may help counteracting vascular aging and its deleterious consequences. Among them, the heptapeptide angiotensin (Ang)-(1–7) has emerged over the last years as a promising candidate with potential pharmacological applications. This review aims to gather evidence demonstrating that Ang-(1–7) arises as an appealing therapeutical target against vascular aging.

3. Angiotensin-(1–7) and the protective branch of the RAS

The RAS is a critical regulator of CV homeostasis and a key player in vascular pathogenesis. Ang II, generated from Ang I as a product of angiotensin-converting enzyme (ACE), is the main bioactive peptide of the RAS and exhibits vasoconstrictile, pro-inflammatory and pro-oxidant actions by activating AT1 receptors [7]. There is evidence for increased vascular expression of Ang II and angiotensin-converting enzyme (ACE) with aging [8,9], while a role for the RAS has been claimed in aged-related CV decline [10].

The heptapeptide Ang-(1–7) is a key player of the so-called protective branch of the RAS and opposes the actions of Ang II mainly by binding the G protein-coupled receptor Mas [11,12]. Ang-(1–7) is generated from Ang II through the action of angiotensin-converting enzyme 2 (ACE2), but also from Ang I via neutral endopeptidase activity [13]. In the CV system, Ang-(1–7) has proven to counterbalance the pro-oxidant, pro-inflammatory, pro-proliferative and vasoconstrictor actions of Ang II [14,15].

Under physiological conditions, the RAS peptides are produced in a balanced manner, whereas both in aging and in chronic diseases, an unbalanced Ang II/Ang-(1–7) ratio has been observed [16–18]. Consistent with this notion, therapies that block Ang II activity and favor Ang-(1–7) production, such as ACE inhibitors and angiotensin receptor blockers (ARBs), reduce risk factors and prevent the decline and impairment of CV function in aged animals and clinical cohorts [19]. Less is known, however, about the ability of the heptapeptide to directly oppose the progeric actions of the RAS or other biological stressors. Over the next sections, the current knowledge of Ang-(1–7) as a protective agent against vascular aging will be reviewed.

4. Ang-(1–7) as a vascular anti-aging peptide

4.1. Vascular cell senescence

In the early 60's, Hayflick and Moorhead defined cellular senescence as a condition where cells permanently lose their ability to proliferate yet remaining metabolically active. The onset of vascular cell senescence is associated with a series of morphological and metabolic disturbances that result in dysfunctional cell homeostasis [20]. Since that very first definition, two main forms of senescence are currently acknowledged: *a*) the one resulting from consecutive cell divisions (replicative senescence) and *b*) a premature form of senescence resulting from the actions of external insults (stress-induced senescence) [21].

Importantly, senescent cells shift to a senescence-associated secretory phenotype (SASP) that releases chemoattractant and pro-inflammatory signals to attract immune cells for the elimination of defective senescent cells by phagocytes [20]. However, when excessive or when vascular senescent cells accumulate, these signals contribute to

create a pro-inflammatory environment, which propagates senescence to neighbouring cells and promotes vascular inflammaging [22].

Although senescence has been comprehensively characterized in cell culture, there is evidence that the senescent phenotype *in vivo* has relevant pathophysiological implications, particularly in the CV system [23]. Indeed, the endothelial senescence phenotype is characterized by typical changes of dysfunctional endothelium, like impaired reactivity with defective relaxation, enhanced pro-inflammatory and pro-coagulant status, remodelling and atherogenesis, among other. Clinically, the accumulation of vascular senescent cells has been well characterized in human atherosclerotic lesions and seems to be critical for the development and progression of the lesion [24]. Therefore, attenuating the induction and accumulation of vascular senescent cells emerges as an innovative and challenging approach for atherosclerosis treatment and prevention.

In this context, Ang-(1–7) has proven to counteract cultured human endothelial cell senescence induced by Ang II via Mas receptors [25]. The heptapeptide attenuates the increase in senescence-associated β -galactosidase (SA- β gal), which reflects the increased lysosomal mass of senescent cells [25]. Ang-(1–7) further reduces total and telomeric DNA damage [25], an early event that signals downstream to provoke growth arrest and senescence [6]. Accordingly, a microarray identified the growth-arrest effector p53 pathway as a main protein cluster influenced by Ang-(1–7) in endothelial cells [26].

Importantly, such a protective *in vitro* action of Ang-(1–7) was equally exerted against endothelial cell senescence induced by a non-RAS stimulus, such as the pro-inflammatory cytokine interleukin-1 β (IL-1 β) [25]. Importantly, IL-1 β has gained relevance in the field of vascular diseases and atherosclerosis after the demonstration by the CANTOS trial that the anti-IL-1 β monoclonal antibody canakinumab reduced atherothrombosis and CV outcomes in humans [27]. The anti-senescence action of Ang-(1–7) has been also shown *in vivo* since the over-expression of p53 observed in the aortas of C57BL6/J mice infused with IL-1 β for 7 days was markedly attenuated by co-infusion of Ang-(1–7) [28]. Overall, this suggests pleiotropic benefits of the Ang-(1–7)/Mas receptor axis beyond Ang II and the RAS and reinforces the potential interest of the heptapeptide for fighting vascular disturbances related to aging.

In addition, Ang-(1–7) may exert beneficial effects against endothelial cell senescence through the attenuation of telomere shortening. The heptapeptide increases the expression and activity of the catalytic subunit of the human telomerase reverse transcriptase (TERT) in cultured human coronary artery endothelial cells [29]. This enzyme is responsible for catalyzing the addition of nucleotides in a TTAGGG sequence to the ends of the chromosome's telomeres [30]. In the microvasculature of coronary artery disease patients, Ang-(1–7)'s protective effects required TERT activity to exert its vasodilator properties [29]. Indeed, TERT has been proposed as a novel therapeutic target against CV diseases [30].

Finally, persistent activation of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) is associated with vascular cell disturbances, including apoptosis and senescence, which in turn promote endothelial dysfunction and atherosclerosis [31–33]. In animal models, aging favors vascular ER alterations that trigger UPR signaling and reduced protective adaptation [34]. In this context, Ang-(1–7) exerts a beneficial effect by ameliorating Ang II-stimulated ER stress and by raising nitric oxide bioavailability [35].

4.2. Inflammation and oxidative stress

Accumulating evidence indicates that aging is associated with a chronic low-level sterile inflammation, termed inflammaging. The modulation of such hyperinflammatory status is emerging as a therapeutical strategy to prevent or delay CV complications and frailty.

While enhanced production of Ang II contributes to vascular inflammation, the activity of the ACE2-Ang-(1–7)/Mas protective

branch of the RAS reduces inflammation in the context of atherosclerosis and chronic renal failure [15, 36, 37]. Indeed, in ApoE knockout mice a deficiency in ACE2 notably accentuates the expression and release of pro-inflammatory markers, including cytokines, adhesion molecules and leukocyte adhesion to the endothelium [38].

Importantly, Ang-(1–7) is able to dampen inflammation beyond the RAS. In lipopolysaccharide (LPS)-treated rats, Ang-(1–7) reduced the plasma levels of IL-6, as well as the production of pro-inflammatory mediators chemokine ligand (CXCL)–1 and macrophage inflammatory protein (MIP)–3 α in the lung [39]. Moreover, Ang-(1–7) was found to downregulate the immune response-related toll-like receptor 4 (TLR4) in vitro [40]. In human endothelial cells, Ang-(1–7) abolishes the auto-activation of IL-1 β release [28]. This is important considering that the pro-inflammatory mediator high sensitivity C-reactive protein, used as a biomarker of CV risk, is now considered as a surrogate marker of IL-1 β tissue activity [27]. Ang-(1–7) has even been proposed as a protective mediator against lung and vascular hyperinflammation in the context of COVID-19 [41,42].

In human VSMC, Ang-(1–7) abrogates the inflammatory effect of RAS-dependent and -independent stimuli through Mas receptors [43]. In response to IL-1 β or LPS, both in vitro and in vivo, Ang-(1–7) inhibited NADPH oxidase activity and the concomitant NF- κ B activation leading to the downregulation of the inducible isoform of NO synthase (iNOS) [39,43]. In fact, iNOS is over-activated in a wide variety of age-related diseases underlying a state of low-grade chronic inflammation, such as atherosclerosis, obesity or diabetic vasculopathy [44]. It is equally upregulated in the human vasculature as we age, even in the absence of evidence for CV diseases [45].

Reactive oxygen species (ROS) act as physiological second messengers in cell signaling, proliferation or differentiation, yet an imbalanced production of oxidant and antioxidant effectors can cause cellular and molecular damage, as occurs in aging [6,46]. Several reports evidenced that long-term ACE inhibition or disruption of AT1 receptor signaling promote longevity in rats and mice [16]. They suggested that longer lifespan was achieved as a consequence of reduced mitochondrial damage and attenuation of oxidative stress. Thus, the Ang-(1–7) capability to attenuate oxidative status may also justify its anti-aging properties. For instance, in mouse models lacking Mas receptors, there is an increase in ROS and downmodulation of endothelial NO production [47, 48]. Ang-(1–7) inhibits the Ang-II-induced ROS production in endothelial cells [49]. Importantly, Ang-(1–7) effectively attenuates the activity of NADPH oxidase, a major source of ROS in vascular disease and aging, in cultured human vascular cells and in vivo animal models [43, 50, 51, 52].

The combination of iNOS-derived NO and certain ROS such as superoxide anions forms an aggressive and cytotoxic species such as peroxynitrite that interacts with cellular macromolecules. In human aging, an increased formation of vascular peroxynitrite has been described [45]. In db/db mice, subcutaneous administration of Ang-(1–7) prevented the progression of kidney damage by reducing oxidative stress as evidenced by a reduced formation of nitrotyrosine residues [53], that are formed by the nitration of both free tyrosine and protein-bound residues in presence of peroxynitrite [54]. Indeed, in patients with chronic kidney disease, circulating Ang-(1–7) levels can be considered as a predictor of reduced extracellular glutathione peroxidase activity, which is essential for the detoxification of hydrogen peroxide radicals [55].

4.3. NLRP3 inflammasome activation

More recently, the sterile over-activation of different players of the innate immune response has been implicated in the development of inflammaging [21]. Supporting the clinical relevance of these findings, epidemiological data suggest an association between elevated inflammatory cytokines and mortality in the elderly [56,57].

In this context, attention has been paid to the nucleotide-binding

oligomerization domain leucine-rich repeat and pyrin domain containing-3 (NLRP3) inflammasome, a large intracellular trimeric protein complex and a crucial sensor of innate immunity. The NLRP3 inflammasome responds and coordinates host defense to different pathogenic and non-pathogenic stressors and danger signals through caspase-1 activation and subsequent secretion of mature pro-inflammatory cytokines IL-1 β and IL-18 and pyroptosis [58].

The over-stimulation of this protein complex, in particular as a response to a range of elements developed in consequence of aging, obesity and other environmental factors, contributes to a chronic state of sterile inflammation and subsequent sequelae [59]. It has also been strongly associated with age-related pathologies such as atherosclerosis, diabetes and related chronic complications [60,61]. Importantly, IL-1 β is a major product of the NLRP3 inflammasome activation [27].

The role of NLRP3 inflammasome in CV events can be attributed to a direct effect on vascular cells which can induce pathological, structural and functional changes in the vasculature. Indeed, both the knockdown of NLRP3 protein and ROS scavengers efficiently prevented the activation of NLRP3 inflammasome complex and pyroptotic human umbilical vein endothelial cells (HUVEC) death [62,63]. IL-1 β has been shown to induce endothelial inflammation illustrated by the over-expression of adhesion molecules and chemokines and the recruitment of leukocytes in HUVEC [25,61], which can be suppressed by the modulation of NLRP3 inflammasome activity [64].

In this context, Ang-(1–7) has revealed itself as an effective inhibitor of NLRP3 inflammasome in the vasculature. Ang-(1–7) was able to mitigate both the priming and activation phases of the NLRP3 inflammasome, as reflected by a lower expression of NLRP3 and pro-IL-1 β proteins as well as diminished NLRP3 inflammasome assembly and mature IL-1 β release [28]. By decreasing ROS formation, a driver of the activation phase of the NLRP3 inflammasome, Ang-(1–7) might reduce the activity of the NLRP3 inflammasome and the release of IL-1 β and IL-18. In fact, Ang-(1–7) shared the actions of MCC950, an NLRP3 inflammasome assembly blocker, in preventing in human endothelial senescence and impaired vascular relaxation induced by IL-1 β [28]. The effect of Ang-(1–7) on the NLRP3 inflammasome has also shown to be beneficial in several non-vascular injuries [65,66]. This, together with other pleiotropic actions of Ang-(1–7) described above, makes of this peptide an attractive target to combat vascular inflammaging and its deleterious consequences.

4.4. Activation of cytoprotective systems

Ang-(1–7) is an activator of different intracellular pathways through which it exerts antitumoral, antiproliferative, antioxidant effects, among other protective actions [15]. In this context, Ang-(1–7) activates protein-tyrosine phosphatase-1 (SHP-1), which in turn inhibits the phosphorylation of p38-mitogen-activated protein kinase (p38MAPK), a member of the MAPK family with great relevance in stress signaling [67]. By stimulating this signaling pathway, Ang-(1–7) has been shown to improve renal vascular function and to counteract the stimulation of matrix metalloproteases involved in vascular remodeling, aneurysm rupture and atherosclerotic plaque instability [67,68]. Moreover, Ang-(1–7) counteracts Ang II-induced signaling in human endothelial cells by reducing the activation of ERK 1/2 MAPK and c-Src protein eventually attenuating NADPH oxidase activity and oxidative stress [12, 69].

Recently, the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) was described as one of the mechanisms by which Ang-(1–7) attenuated premature endothelial senescence triggered by the stressors Ang II and IL-1 β [25]. Nrf2 is an evolutionary conserved transcription factor that protects against oxidative stress and inflammation by promoting the expression of detoxifying enzymes [70]. Nrf2 activity decreases with age and its deficiency is associated with human progeric syndromes [70,71]. In particular, the induction of heme oxygenase-1 (HO-1), a member of the heatshock family of proteins which produces

carbon monoxide, biliverdin and iron, appeared as a main mediator of the antisenescence and vasorelaxant properties of Ang-(1–7) [25]. Indeed, through its antioxidant properties HO-1 can increase NO bioavailability in endothelial cells, thus improving vascular relaxation and endothelial function [72,73].

Moreover, Ang-(1–7) was shown to promote the activation of the Nrf2/HO-1 axis in human endothelial cells by the induction of klotho protein [25]. Klotho was identified as an anti-aging factor since mice deficient of this protein exhibited patterns of premature aging, including renal dysfunction, vascular calcification, atherosclerosis, and a reduced lifespan [74,75].

4.5. Vascular reactivity

The endothelial layer is fundamental for maintaining the vessels homeostasis. In physiological conditions, endothelial cells release a number of substances or factors that tightly control vascular function. When this fine equilibrium is disrupted, endothelial dysfunction takes place, which favours a pro-contractile, pro-inflammatory, pro-oxidant, pro-proliferative and pro-coagulant environment. Endothelial dysfunction, and particularly the loss of vasorelaxant capacity, is an early functional marker of vascular disease and predicts major causes of vascular morbidity and mortality [76].

When associated to aging, endothelial dysfunction is clearly multifactorial, with different pathophysiological mechanisms contributing to the deterioration of vascular function and reactivity. Among them, a major player is the loss of vasorelaxant factors, and particularly of NO bioavailability. Several groups, including ours, have described that impaired endothelium-dependent vasodilatation takes place in both aged animal models and elderly subjects [45, 77, 78, 79]. The loss of vasorelaxant factors is paralleled by an over-activity of cyclooxygenase-derived vasoconstrictile and pro-inflammatory factors, such as thromboxane A₂, which has been associated with vascular inflammation, aging and other progeric conditions such as diabetes mellitus [2,80].

In this regard, Ang-(1–7) may exert a protective effect as it promotes both *ex vivo* and *in vivo* vascular relaxation and yields an additional supply of NO [11, 28, 48]. In endothelial cells, Ang-(1–7) activates phosphatidylinositol-4,5-bisphosphate 3-kinase / protein kinase B (PI3K/Akt), which in turn increases endothelial nitric oxide synthase activity and promotes the release of NO with vasodilatory, anti-inflammatory, anti-oxidant and anti-aggregant properties in the vasculature [11,81].

Moreover, Ang-(1–7)/Mas may induce the release of other vasodilators like prostanoids or endothelium-derived hyperpolarizing factor by phospholipase A₂ activation or other intracellular pathways [82]. Interestingly, the anti-aging protein klotho, which is up-regulated in endothelial cells by Ang-(1–7), equally exerts vasodilatory actions [25, 83].

4.6. Fibrosis and calcification

Vascular remodelling mediated by fibrosis and calcification of the blood vessels is a hallmark of vascular dysfunction associated with aging, as much as it is with progeric pathologies such as chronic kidney disease or diabetes mellitus [84]. Eventually, calcification leads to vascular stiffness and exerts a profound negative influence on blood vessel functionality [85]. When the arteries become stiffer, vascular compliance or distensibility is reduced, being a key feature of the aged vessel, since calcified arterial walls are unable to dilate or contract in response to physiological stimuli, resulting in disturbed regulation of blood flow and endothelial dysfunction. Globally, vascular calcification is associated with increased mortality and morbidity due to CV disease. In fact, arterial stiffness is an independent predictor of CV risk and a *sine qua non* condition for the development of systolic hypertension, a characteristic hypertensive condition in the elderly [86].

Ang-(1–7) has demonstrated to be an efficient inhibitor of fibrosis and calcification within the vasculature by counteracting the deleterious effects of Ang II. Its anti-fibrotic actions comprise the modulation of fibroblast density as well as the inhibition of fibrogenic pathways and the production of extracellular matrix proteins such as collagen and matrix metalloproteinases [87]. In fact, loss of ACE2 has been shown to induce Ang II-mediated expression of the fibrosis-associated genes transforming growth factor- β , connective tissue growth factor, procollagen type I and procollagen type III [87,88]. Besides, Ang-(1–7) halts vascular calcification, as demonstrated on VSMC *in vitro* [89], as well as in rat calcified aortas *in vivo* [90].

4.7. Angiogenesis and repair

In addition to the aforementioned mechanisms by which Ang-(1–7) has demonstrated to mitigate vascular aging, there are other effects of this peptide that contribute to its anti-aging actions. Ang-(1–7) administered to aged mice induces migration and proliferation of bone marrow-derived hematopoietic stem/progenitor cells (HSPCs), which are crucial to vascular regeneration [91]. Ang-(1–7) stimulates mobilization of these HSPCs in response to ischemia and through this effect, promotes vascular regeneration on old mice [91]. Diabetes, a forceful driver of early vascular aging, has been reported to alter the ACE/ACE2 balance on HSPCs resulting on lower levels of Ang-(1–7) and impaired regenerative function, which was restored by genetic transfer of ACE2 enzyme [91].

Besides, Ang-(1–7) activates therapeutic angiogenesis, a crucial process for tissue regeneration. In particular, infusion of Ang-(1–7) stimulated brain endothelial cell proliferation and angiogenesis by increasing NO within the endothelial cells, decreasing infarct volume and neurological deficits of rats subjected to permanent middle cerebral artery occlusion [92]. Furthermore, Ang-(1–7) has proven to counteract the pro-apoptotic effects of Ang II in cerebral endothelial cells [93], in addition to the cytoprotective mechanisms enumerated in Section 4.4. According to this, in a context of vascular aging with an imbalance of Ang II/Ang-(1–7) levels, endothelial brain cells will exhibit greater dysfunction and impaired angiogenesis.

5. Influence of gender and aging on the protective branch of the RAS

The expression and activity of the tissue and vascular components of the protective branch of the RAS is itself influenced by aging and markedly determined by sexual hormones. Hence, the protective capacity of endogenous Ang-(1–7) will vary dependent on factors such as age and sex.

In clinical populations, plasma Ang-(1–7) levels are higher in young healthy women versus men [94]. However, menopause and the decline of sex hormones negatively influences the expression of the components of the protective branch of the RAS in women, with Ang-(1–7) plasma levels being lower in post-menopausal than in premenopausal women [95]. These observations are in line with the capacity of estrogens to upregulate the protective Ang-(1–7)-ACE2-Mas-AT2 pathway [96], opposite to testosterone that favors the activation of the Ang-II-ACE-AT1 branch of the RAS [97]. In cultured human endothelial cells, estradiol induces the synthesis and release of Ang-(1–7) [98], while estradiol deficiency is associated with defective vascular responsiveness to Ang-(1–7) and vascular dysfunction in female animal models [99,100]. Thus, and despite the need of additional studies, the decline of the protective branch of the RAS, also at the vascular level, seems to be one relevant factor contributing to vascular impairment and arterial stiffness in post-menopausal women.

Overall, Ang-(1–7) levels have been reported to decay during aging [100]. In rat and mice models, older animals exhibit higher ACE activity but lower ACE2 activity as compared to young ones in different tissues including the vasculature [101,102]. Similar observations have been

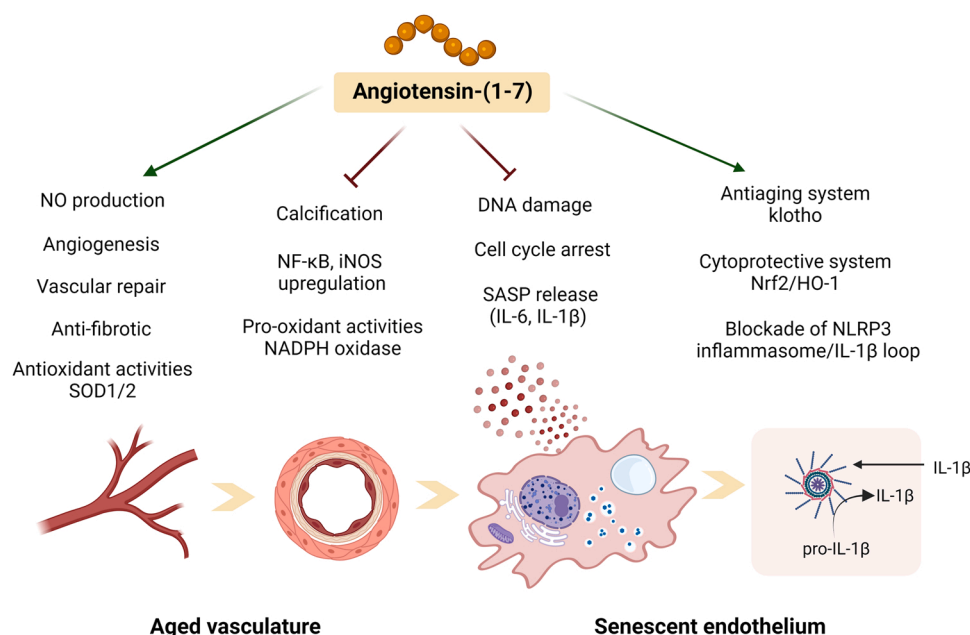


Fig. 1. Graphical abstract summarizing the main mechanisms by which Ang-(1–7) exerts protective effects against vascular aging. Ang-(1–7) mitigates the senescence of vascular cells and the secretion of pro-inflammatory factors by inhibiting NF-κB and NADPH oxidase and NLRP3 inflammasome activation while up-regulating anti-inflammatory and cytoprotective systems, such as the Nrf2-HO-1 axis or the anti-aging protein klotho, among other. Ang-(1–7) further acts on other hallmarks of vascular aging such as the loss of NO and endothelial dysfunction, fibrosis and calcification, and promoted protective angiogenesis and vascular repair. IL: interleukin; HO-1, heme oxygenase 1; NF-κB, nuclear factor-κB; Nrf2, nuclear factor erythroid 2-related factor 2; SASP, senescence-associated secretory phenotype; SOD, superoxide dismutase.

made in human aortic tissue in which the ACE/Ang II/AT1 axis preponderates with age [103]. Indeed, this predominant axis appears to play a key local role in experimental age-dependent endothelial dysfunction [104]. Also in support of a decay of the RAS protective axis with age, the levels of bradykinin receptor 2, which can form heterodimers with Mas receptors to mediate the protective actions of Ang-(1–7), also decline with age [105].

Moreover, the aging-dependent loss of the protective branch of the RAS seems to be particularly apparent in certain conditions, such as overweight and obesity [100], which are considered as progeric diseases exhibiting accelerated vascular aging. However, young females, but not older ones, would benefit from protection against hypertension due to higher ACE2 levels and Ang-(1–7) generation in the adipose tissue [106].

Globally, these and other pieces of evidence underpin that as we age there are clinical or physiological situations implying a loss of balance between the main two RAS branches in detriment of the protective one. In such situations, the pharmacological supplementation with Ang-(1–7) might be of particular benefit.

6. Future perspectives

With the extension of life expectancy and the rising percentage of older individuals in the general population, understanding why vascular aging results in progressively higher susceptibility to chronic morbidity, disability, and frailty has become a public health priority. There is still limited clinical evidence demonstrating the CV therapeutic potential of Ang-(1–7) in humans, in part due to regulatory limitations and the need of improved formulations and better safety assessments. However, recent evidence supports a promising therapeutic potential for the heptapeptide as a novel therapeutic agent for improving insulin sensitivity and preserving endothelial function in human obesity [107].

Hence, the ACE2/Ang-(1–7)/Mas axis, and the therapeutic strategies oriented to increase its activation within the organism, arise as extremely helpful treatments to delay vascular damage, premature aging and its associated complications. Furthermore, preservation of vascular function in aging should not only reduce deaths and disabilities secondary to CV events but also could attenuate other negative aspects of the aging process that lead to loss of function and/or disability, such as motor capacity and cognitive frailty. More attention needs to be paid to sex and aging as biological variables influencing the response to

pharmacological treatments including those aimed at reinforcing the protective action of Ang-(1–7).

While more evidence in humans is needed, there are several ongoing clinical trials to test Ang-(1–7) treatment in CV disease, as well as in other settings such as cancer or COVID-19, where it holds great promise. A phase II clinical trial for metastatic sarcoma has already been completed and, although it did not show efficacy for this particular pathology, it demonstrated safety in humans and provided useful pharmacokinetic data [108]. While the role of Ang-(1–7) in cancer remains controversial, it could help attenuating the endothelial cell senescence and the vascular toxicity induced by anti-cancer drugs such as doxorubicin [109].

Given all the beneficial effects hereby reported for Ang-(1–7), the treatment with the heptapeptide or other pharmacological interventions to stimulate its production might be implemented in the near future for the treatment of vascular aging and related diseases, probably in combination with current medications (Fig. 1).

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References

- [1] S. Costantino, F. Paneni, F. Cosentino, Ageing, metabolism and cardiovascular disease, *J. Physiol.* 594 (2016) 2061–2073.
- [2] Z. Ungvari, S. Tarantini, A.J. Donato, V. Galvan, A. Csiszar, Mechanisms of vascular aging, *Circ. Res.* 123 (2018) 849–867.
- [3] S.S. Najjar, A. Scuteri, E.G. Lakatta, Arterial aging: is it an immutable cardiovascular risk factor? Hypertension. 46 (2005) 454–462.
- [4] J. Sabbatini, D. Ramini, A. Giuliani, R. Recchioni, L. Spazzafumo, F. Olivieri, Connecting vascular aging and frailty in Alzheimer's disease, *Mech. Ageing* 195 (2021), 111444.
- [5] V. Kotsis, S. Stabouli, I. Karafilis, P. Nilsson, Early vascular aging and the role of central blood pressure, *J. Hypertens.* 29 (2011) 1847–1853.
- [6] C. López-Otín, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging, *Cell* 153 (2013) 1194–1217.

- [7] A.C. Montezano, A. Nguyen Dinh Cat, F.J. Rios, R.M. Touyz, Angiotensin II and vascular injury, *Curr. Hypertens. Rep.* 16 (2014) 431.
- [8] S. Rajagopalan, R. Brook, R.H. Mehta, M. Supiano, B. Pitt, Effect of losartan in aging-related endothelial impairment, *Am. J. Cardiol.* 89 (2002) 562–566.
- [9] M. Wang, G. Takagi, K. Asai, R.G. Resuello, F.F. Natividad, D.E. Vatner, S. F. Vatner, E.G. Lakatta, Aging increases aortic MMP-2 activity and angiotensin II in nonhuman primates, *Hypertension* 41 (2003) 1308–1316.
- [10] M. Mogi, Effect of renin-angiotensin system on senescence, *Geriatr. Gerontol. Int.* 20 (2020) 520–525.
- [11] R.A. Santos, A.C. Simoes e Silva, C. Maric, D.M. Silva, R.P. Machado, I. de Buhr, S. Heringer-Walther, S.V. Pinheiro, M.T. Lopes, M. Bader, E.P. Mendes, V. S. Lemos, M.J. Campagnole-Santos, H.P. Schultheiss, R. Speth, T. Walther, Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 8258–8263.
- [12] W.O. Sampaio, C. Henrique de Castro, R.A. Santos, E.L. Schiffrin, R.M. Touyz, Angiotensin-(1-7) counterregulates angiotensin II signaling in human endothelial cells, *Hypertension* 50 (2007) 1093–1098.
- [13] L.T. McCollum, P.E. Gallagher, E. Ann Tallant, Angiotensin-(1-7) attenuates angiotensin II-induced cardiac remodeling associated with upregulation of dual-specificity phosphatase 1, *Am. J. Physiol. Heart Circ. Physiol.* 302 (2012) H801–H810.
- [14] D.G. Passos-Silva, E. Brandan, R.A. Santos, Angiotensins as therapeutic targets beyond heart disease, *Trends Pharmacol. Sci.* 36 (2015) 310–320.
- [15] R.A.S. Santos, W.O. Sampaio, A.C. Alzamora, D. Motta-Santos, N. Alenina, M. Bader, M.J. Campagnole-Santos, The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7), *Physiol. Rev.* 98 (2018) 505–553.
- [16] A.J. Miller, S.S. Bingaman, D. Mehay, D. Medina, A.C. Arnold, Angiotensin-(1-7) improves integrated cardiometabolic function in aged mice, *Int. J. Mol. Sci.* 21 (2020) 5131.
- [17] P. Srivastava, S. Badhwar, D.S. Chandran, A.K. Jaryal, V.P. Jyotsna, K.K. Deepak, Imbalance between Angiotensin II - Angiotensin (1-7) system is associated with vascular endothelial dysfunction and inflammation in type 2 diabetes with newly diagnosed hypertension, *Diabetes Metab. Syndr.* 13 (2019) 2061–2068.
- [18] S.H. Sousa-Santos, J.M. Oliveira Andrade, Angiotensin 1-7: a peptide for preventing and treating metabolic syndrome, *Peptides* 59 (2014) 34–41.
- [19] M.A. Mohamed, M.R. Weir, Renin angiotensin system inhibition in the older person: a review, *Clin. Geriatr. Med.* 25 (2009) 245–257.
- [20] J.D. Erusalimsky, Vascular endothelial senescence: from mechanisms to pathophysiology, *J. Appl. Physiol.* 106 (2009) 326–332.
- [21] I. Ben-Porath, R.A. Weinberg, When cells get stressed: an integrative view of cellular senescence, *J. Clin. Invest.* 113 (2004) 8–13.
- [22] L. Ferrucci, E. Fabbri, Inflammaging: chronic inflammation in ageing, cardiovascular disease, and frailty, *Nat. Rev. Cardiol.* 15 (2018) 505–522.
- [23] J.D. Erusalimsky, D.J. Kurz, Cellular senescence in vivo: its relevance in ageing and cardiovascular disease, *Exp. Gerontol.* 40 (2005) 634–642.
- [24] T. Minamino, I. Komuro, Vascular cell senescence: contribution to atherosclerosis, *Circ. Res.* 100 (2007) 15–26.
- [25] A. Romero, Á. San Hipólito-Luengo, L.A. Villalobos, S. Vallejo, I. Valencia, P. Michalska, N. Pajuelo-Lozano, I. Sánchez-Pérez, R. León, J.L. Bartha, M.J. Sanz, J.D. Erusalimsky, C.F. Sánchez-Ferrer, T. Romacho, C. Peiró, The angiotensin-(1-7)/Mas receptor axis protects from endothelial cell senescence via klotho and Nrf2 activation, *Aging Cell* 18 (2019), e12913.
- [26] C. Meinert, F. Kohse, I. Böhme, F. Gembardt, A. Tetzner, T. Wieland, B. Greenberg, T. Walther, Further intracellular proteins and signaling pathways regulated by angiotensin-(1-7) in human endothelial cells, *Data Brief.* 10 (2016) 354–363.
- [27] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, F. Fonseca, J. Nicolau, W. Koenig, S.D. Anker, J. Kastelein, J. H. Cornel, P. Pais, D. Pella, J. Genest, R. Cifkova, A. Lorenzatti, T. Forster, Z. Kobalava, L. Vida-Simiti, M. Flather, H. Shimokawa, H. Ogawa, M. Dellborg, P. Rossi, R. Troquay, P. Libby, R.J. Glynn, G. CANTOS Trial, Antiinflammatory therapy with canakinumab for atherosclerotic disease, *New Engl. J. Med.* 377 (2017) 1119–1131.
- [28] A. Romero, P. Dongil, I. Valencia, S. Vallejo, Á.S. Hipólito-Luengo, G. Díaz-Araya, J.L. Bartha, M.M. González-Arlanzón, F. Rivilla, F. de la Cuesta, C.F. Sánchez-Ferrer, C. Peiró, Pharmacological blockade of NLRP3 inflammasome/IL-1 β -positive loop mitigates endothelial cell senescence and dysfunction, *Aging Dis.* 13 (2022) 284–297.
- [29] M.J. Durand, N.S. Zinkevich, M. Riedel, D.D. Gutterman, V.L. Nasci, V.K. Salato, J.B. Hijawi, C.F. Reuben, P.E. North, A.M. Beyer, Vascular actions of angiotensin 1-7 in the human microcirculation: novel role for telomerase, *Arterioscler. Thromb. Vasc. Biol.* 36 (2016) 1254–1262.
- [30] J. Hoffmann, G. Richardson, J. Haendeler, J. Altschmied, V. Andrés, I. Spyridopoulos, Telomerase as a therapeutic target in cardiovascular disease, *Arterioscler. Thromb. Vasc. Biol.* 41 (2021) 1047–1061.
- [31] M.R. Hamczyk, R. Villa-Bellota, V. Quesada, P. Gonzalo, S. Vidak, R.M. Nevado, M.J. Andrés-Manzano, T. Misteli, C. López-Otín, V. Andrés, Progerin accelerates atherosclerosis by inducing endoplasmic reticulum stress in vascular smooth muscle cells, *EMBO Mol. Med.* 11 (2019), e9736.
- [32] S.M. Kang, H.S. Jung, M.J. Kwon, S.H. Lee, J.H. Park, Effects of anagliptin on the stress induced accelerated senescence of human umbilical vein endothelial cells, *Ann. Transl. Med.* 9 (2021) 750.
- [33] V. Sepúlveda-Fragoso, B. Alexandre-Santos, A.C.P. Salles, A.B. Proença, A.P. de Paula Alves, M. Vázquez-Carrera, A. Nóbrega, E. Frantz, D.C. Magliano, Crosstalk between the renin-angiotensin system and the endoplasmic reticulum stress in the cardiovascular system: lessons learned so far, *Life Sci.* 284 (2021), 119919.
- [34] Y. Zhou, X. Wan, K. Seidel, M. Zhang, J.B. Goodman, F. Seta, N. Hamburg, J. Han, Aging and Hypercholesterolemia Differentially Affect the Unfolded Protein Response in the Vasculature of ApoE(-/-) Mice, *J. Am. Heart Assoc.* 10 (2021), e020441.
- [35] D. Murugan, Y.S. Lau, C.W. Lau, M.R. Mustafa, Y. Huang, Angiotensin 1-7 protects against angiotensin ii-induced endoplasmic reticulum stress and endothelial dysfunction via mas receptor, *PLoS One* 10 (2015), e0145413.
- [36] STARSurg Collaborative, Association between peri-operative angiotensin-converting enzyme inhibitors and angiotensin-2 receptor blockers and acute kidney injury in major elective non-cardiac surgery: a multicentre, prospective cohort study, *Anaesthesia*. 73 (2018) 1214–1222.
- [37] M.H.M. Yousif, G.S. Dhaunsi, B.M. Makki, B.A. Qabazard, S. Akhtar, I.F. Benter, Characterization of Angiotensin-(1-7) effects on the cardiovascular system in an experimental model of Type-1 diabetes, *Pharmacol. Res.* 66 (2012) 269–275.
- [38] M.C. Thomas, R.J. Pickering, D. Tsorotes, A. Koitka, K. Sheehy, S. Bernardi, B. Toffoli, T.P. Nguyen-Huu, G.A. Head, Y. Fu, J. Chin-Dusting, M.E. Cooper, C. Tikellis, Genetic Ace2 deficiency accentuates vascular inflammation and atherosclerosis in the ApoE knockout mouse, *Circ. Res.* 107 (2010) 888–897.
- [39] H.J. Tsai, C.C. Shih, K.Y. Chang, M.H. Liao, W.J. Liaw, C.C. Wu, C.M. Tsao, Angiotensin-(1-7) treatment blocks lipopolysaccharide-induced organ damage, platelet dysfunction, and IL-6 and nitric oxide production in rats, *Sci. Rep.* 11 (2021) 610.
- [40] Y. Wang, G. Wang, L. Cui, R. Liu, H. Xiao, C. Yin, Angiotensin 1-7 ameliorates caerulein-induced inflammation in pancreatic acinar cells by downregulating Toll-like receptor 4/nuclear factor- κ B expression, *Mol. Med. Rep.* 17 (2017) 3511–3518.
- [41] C. Peiró, S. Moncada, Substituting angiotensin-(1-7) to prevent lung damage in SARS-CoV-2 infection? *Circulation* 141 (2020) 1665–1666.
- [42] G.S. Magalhães, M.D.G. Rodrigues-Machado, D. Motta-Santos, M.J. Campagnole-Santos, R.A.S. Santos, Activation of Ang-(1-7)/Mas Receptor Is a Possible Strategy to Treat Coronavirus (SARS-CoV-2) Infection, *Front. Physiol.* 11 (2020) 730.
- [43] L.A. Villalobos, Á. San Hipólito-Luengo, M. Ramos-González, E. Cercas, S. Vallejo, A. Romero, T. Romacho, R. Carraro, C.F. Sánchez-Ferrer, C. Peiró, The angiotensin-(1-7)/mas axis counteracts angiotensin ii-dependent and -independent pro-inflammatory signaling in human vascular smooth muscle cells, *Front. Pharmacol.* 7 (2016) 482.
- [44] G.H. Oliveira-Paula, R. Lacchini, J.E. Tanus-Santos, Inducible nitric oxide synthase as a possible target in hypertension, *Curr. Drug Targets* 15 (2014) 164–174.
- [45] L. Rodríguez-Mañas, M. El-Assar, S. Vallejo, P. López-Dóriga, J. Solís, R. Petidier, M. Montes, J. Nevado, M. Castro, C. Gómez-Guerrero, C. Peiró, C.F. Sánchez-Ferrer, Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation, *Aging Cell* 8 (2009) 226–238.
- [46] B.L. Tan, M.E. Norhaizan, W. Liew, R.H. Sulaiman, Antioxidant and oxidative stress: a mutual interplay in age-related diseases, *Front. Pharmacol.* 16 (2018) 1162.
- [47] L.A. Rabelo, P. Xu, M. Todiras, W.O. Sampaio, J. Buttgerit, M. Bader, R. A. Santos, N. Alenina, Ablation of angiotensin (1-7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction, *J. Am. Soc. Hypertens.* 2 (2008) 418–424.
- [48] C. Peiró, S. Vallejo, F. Gembardt, V. Azcutia, S. Heringer-Walther, L. Rodríguez-Mañas, H.P. Schultheiss, C.F. Sánchez-Ferrer, T. Walther, Endothelial dysfunction through genetic deletion or inhibition of the G protein-coupled receptor Mas: a new target to improve endothelial function, *J. Hypertens.* 25 (2007) 2421–2425.
- [49] A.H. Polizio, M.M. Gironacci, M.L. Tomaro, C. Peña, Angiotensin-(1-7) blocks the angiotensin II-stimulated superoxide production, *Pharmacol. Res.* 5 (2007) 86–90.
- [50] I.F. Benter, M.H. Yousif, G.S. Dhaunsi, J. Kaur, M.C. Chappell, D.I. Diz, Angiotensin-(1-7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats, *Am. J. Nephrol.* 28 (2008) 25–33.
- [51] W.Y. Pai, W.Y. Lo, T. Hsu, C.T. Peng, H.J. Wang, Angiotensin-(1-7) inhibits thrombin-induced endothelial phenotypic changes and reactive oxygen species production via NADPH oxidase 5 downregulation, *Front. Physiol.* 8 (2017) 994.
- [52] F. Zhang, X. Ren, M. Zhao, B. Zhou, Y. Han, Angiotensin-(1-7) abrogates angiotensin II-induced proliferation, migration and inflammation in VSMCs through inactivation of ROS-mediated PI3K/Akt and MAPK/ERK signaling pathways, *Sci. Rep.* 6 (2016) 3462.
- [53] [48] A.M. Papinska, K.E. Rodgers, Long-term administration of angiotensin (1-7) to db/db mice reduces oxidative stress damage in the kidneys and prevents renal dysfunction, *Oxid. Med. Cell. Longev.* 2018 (2018), 1841046.
- [54] M. Bandookwala, P. Sengupta, 3-Nitrotyrosine: a versatile oxidative stress biomarker for major neurodegenerative diseases, *Int. J. Neurosci.* 130 (2020) 1047–1062.
- [55] C. Shi, K. Lu, H. Xia, P. Zhang, B. Zhang, Alteration and association between serum ACE2/ angiotensin(1-7)/Mas axis and oxidative stress in chronic kidney disease, *Medicine* 99 (2020), e2194.
- [56] T.B. Harris, L. Ferrucci, R.P. Tracy, M.C. Corti, S. Wacholder, Jr Ettinger WH, H. Heimovitz, H.J. Cohen, R. Wallace Jr., Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly, *Am. J. Med.* 106 (1999) 506–512.
- [57] S. Volpato, J.M. Guralnik, L. Ferrucci, J. Balfour, P. Chaves, L.P. Fried, T. B. Harris, Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study, *Circulation* 103 (2001) 947–953.
- [58] Y. He, H. Hara, G. Núñez, Mechanism and regulation of NLRP3 inflammasome activation, *Trends Biochem. Sci.* 41 (2016) 1012–1021.

- [59] A.K. Meyers, X. Zhu, The NLRP3 inflammasome: metabolic regulation and contribution to inflammaging, *Cells* 9 (2020) 1808.
- [60] S. Menini, C. Iacobini, M. Vitale, G. Pugliese, The inflammasome in chronic complications of diabetes and related metabolic disorders, *Cells* 9 (2020) 1812.
- [61] A. Grebe, F. Hoss, E. Latz, NLRP3 inflammasome and the IL-1 pathway in atherosclerosis, *Circ. Res.* 122 (2018) 1722–1740.
- [62] H. Chen, Y. Lu, Z. Cao, Q. Ma, H. Pi, Y. Fang, Z. Yu, H. Hu, Z. Zhou, Cadmium induces NLRP3 inflammasome-dependent pyroptosis in vascular endothelial cells, *Toxicol. Lett.* 246 (2016) 7–16.
- [63] C. Jiang, L. Jiang, Q. Li, X. Liu, T. Zhang, L. Dong, T. Liu, L. Liu, G. Hu, X. Sun, L. Jiang, Acrolein induces NLRP3 inflammasome-mediated pyroptosis and suppresses migration via ROS-dependent autophagy in vascular endothelial cells, *Toxicology* 410 (2018) 26–40.
- [64] Z. Wan, Y. Fan, X. Liu, J. Xue, Z. Han, C. Zhu, X. Wang, NLRP3 inflammasome promotes diabetes-induced endothelial inflammation and atherosclerosis, *Diabetes Metab. Syndr.* 12 (2019) 1931–1942.
- [65] H. Huang, J. Wang, Z. Liu, F. Gao, The angiotensin-converting enzyme 2/angiotensin (1-7)/mas axis protects against pyroptosis in LPS-induced lung injury by inhibiting NLRP3 activation, *Arch. Biochem. Biophys.* 693 (2020), 108562.
- [66] S.M. Cai, R.Q. Yang, Y. Li, Z.W. Ning, L.L. Zhang, G.S. Zhou, W. Luo, D.H. Li, Y. Chen, M.X. Pan, X. Li, Angiotensin-(1-7) improves liver fibrosis by regulating the NLRP3 inflammasome via redox balance modulation, *Antioxid. Redox Signal.* 24 (2016) 795–812.
- [67] E. Gava, A. Samad-Zadeh, J. Zimpelmann, N. Bahramifarid, G.T. Kitten, R. A. Santos, R.M. Touyz, K.D. Burns, Angiotensin-(1-7) activates a tyrosine phosphatase and inhibits glucose-induced signalling in proximal tubular cells, *Nephrol. Dial. Transplant.* 24 (2009) 1766–1773.
- [68] F. Zhang, S. Li, J. Song, J. Liu, Y. Cui, H. Chen, Angiotensin-(1-7) regulates angiotensin II-induced matrix metalloproteinase-8 in vascular smooth muscle cells, *Atherosclerosis* 261 (2017) 90–98.
- [69] A.C. Da Costa Gonçalves, R. Leite, R.A. Fraga-Silva, S.V. Pinheiro, A.B. Reis, F. M. Reis, R.M. Touyz, R.C. Webb, N. Alenina, M. Bader, R.A. Santos, Evidence that the vasodilator angiotensin-(1-7)-Mas axis plays an important role in erectile function, *Am. J. Physiol. Heart Circ. Physiol.* 293 (2007) H2588–H2596.
- [70] D. Kloska, A. Kopacz, A. Piechota-Polanczyk, W.N. Nowak, J. Dulak, A. Jozkowicz, A. Grochot-Przeczek, Nrf2 in aging – focus on the cardiovascular system, *Vasc. Pharmacol.* 112 (2019) 42–53.
- [71] C.J. Schmidlin, M.B. Dodson, L. Madhavan, D.D. Zhang, Redox regulation by NRF2 in aging and disease, *Free Radic. Biol. Med.* 134 (2019) 702–707.
- [72] A. Loboda, M. Damulewicz, E. Pyza, A. Jozkowicz, J. Dulak, Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism, *Cell. Mol. Life Sci.* 73 (2016) 3221–3247.
- [73] W. Luo, Y. Wang, H. Yang, C. Dai, H. Hong, J. Li, Z. Liu, Z. Guo, X. Chen, P. He, Z. Li, F. Li, J. Jiang, P. Liu, Z. Li, Heme oxygenase-1 ameliorates oxidative stress induce endothelial senescence via regulating endothelial nitric oxide synthase activation and coupling, *Aging* 10 (2018) 1722–1744.
- [74] M. Kuro-o, Y. Matsumura, H. Aizawa, H. Kawaguchi, T. Suga, T. Utsugi, Y. Ohyama, M. Kurabayashi, T. Kaname, E. Kume, H. Iwasaki, A. Iida, T. Shirakida, S. Nishikawa, R. Nagai, Y.I. Nabeshima, Mutation of the mouse klotho gene leads to a syndrome resembling ageing, *Nature* 390 (1997) 45–51.
- [75] P. Buendía, J. Carracedo, S. Soriano, J.A. Madueño, A. Ortiz, A. Martín-Malo, P. Aljama, R. Ramírez, Klotho Prevents NFκB Translocation and Protects Endothelial Cell From Senescence Induced by Uremia, *J. Gerontol. A Biol. Sci. Med. Sci.* 70 (2015) 1198–1209.
- [76] H.F. Galley, N.R. Webster, Physiology of the endothelium, *Br. J. Anaesth.* 93 (2004) 105–113.
- [77] R.L. Matz, R. Andriantsitohaina, Age-related endothelial dysfunction: potential implications for pharmacotherapy, *Drugs Aging* 20 (2003) 527–550.
- [78] R.P. Brandes, I. Fleming, R. Busse, Endothelial aging, *Cardiovasc. Res.* 66 (2005) 286–294.
- [79] N. Toda, Age-related changes in endothelial function and blood flow regulation, *Pharmacol. Ther.* 133 (2012) 159–176.
- [80] M. Féletou, Y. Huang, P.M. Vanhoutte, Endothelium-mediated control of vascular tone: COX-1 and COX-2 products, *Br. J. Pharmacol.* 164 (2011) 894–912.
- [81] A. Sobrino, S. Vallejo, S. Novella, M. Lázaro-Franco, A. Mompeón, C. Bueno-Betf, T. Walther, C. Sánchez-Ferrer, C. Peiró, C. Hermenegildo, Mas receptor is involved in the estrogen-receptor induced nitric oxide-dependent vasorelaxation, *Biochem. Pharmacol.* 129 (2013) 67–72.
- [82] M. Bader, N. Alenina, M. Andrade-Navarro, R. Santos, Mas and its related G protein-coupled receptors, *MRGPRs Pharmacol. Rev.* 66 (2014) 1080–1105.
- [83] Y. Saito, T. Yamagishi, T. Nakamura, Y. Ohyama, H. Aizawa, T. Suga, Y. Matsumura, H. Masuda, M. Kurabayashi, M. Kuro-o, Y. Nabeshima, R. Nagai, Klotho protein protects against endothelial dysfunction, *Biochem. Biophys. Res. Commun.* 248 (1998) 324–329.
- [84] A. Ndiip, F.L. Wilkinson, E.B. Jude, A.J. Boulton, M.Y. Alexander, RANKL-OPG and RAGE modulation in vascular calcification and diabetes: novel targets for therapy, *Diabetologia* 57 (2014) 2251–2260.
- [85] C.M. Giachelli, Vascular calcification mechanisms, *J. Am. Soc. Nephrol.* 15 (2004) 2959–2964.
- [86] P. Greenland, R.O. Bonow, B.H. Brundage, M.J. Budoff, M.J. Eisenberg, S. M. Grundy, M.S. Lauer, W.S. Post, P. Raggi, R.F. Redberg, G.P. Rodgers, L. J. Shaw, A.J. Taylor, W.S. Weintraub, R.A. Harrington, J. Abrams, J.L. Anderson, E.R. Bates, C.L. Grines, M.A. Hlatky, R.C. Lichtenberg, J.R. Lindner, G.M. Pohost, R.S. Schofield, Jr Shubrooks SJ, J.H. Stein, C.M. Tracy, R.A. Vogel, D.J. Westley, T. American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the Expert Consensus Document on Electron Beam Computed, P. Society of Atherosclerosis Imaging and, T. Society of Cardiovascular Computed, ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography), *Circulation* 115 (2007) 402–426.
- [87] Z. Zhang, L. Chen, J. Zhong, P. Gao, G.Y. Oudit, ACE2/Ang-(1-7) signaling and vascular remodeling, *Sci. China Life Sci.* 57 (2014) 802–808.
- [88] B. Song, Z.Z. Zhang, J.C. Zhong, X.Y. Yu, G.Y. Oudit, H.Y. Jin, L. Lu, Y.L. Xu, Z. Kassiri, W.F. Shen, P.J. Gao, D.L. Zhu, Loss of angiotensin-converting enzyme 2 exacerbates myocardial injury via activation of the CTGF-fractalkine signaling pathway, *Circ. J.* 77 (2013) 2997–3006.
- [89] H.Y. Bai, B.S. Shan, Y.N. Jiang, The protective effects of renin-angiotensin system components on vascular calcification, *J. Hum. Hypertens.* 35 (2021) 410–418.
- [90] Y.B. Sui, J.R. Chang, W.J. Chen, L. Zhao, B.H. Zhang, Y.R. Yu, C.S. Tang, X.H. Yin, Y.F. Qi, Angiotensin-(1-7) inhibits vascular calcification in rats, *Peptides* 42 (2013) 25–34.
- [91] S. Joshi, K. Chittimalli, J. Jahan, G. Vasam, Y.P. Jarajapu, ACE2/ACE imbalance and impaired vasoreparative functions of stem/progenitor cells in aging, *Geroscience* 43 (2021) 1423–1436.
- [92] T. Jiang, J.T. Yu, X.C. Zhu, Q.Q. Zhang, M.S. Tan, L. Cao, H.F. Wang, J. Lu, Q. Gao, Y.D. Zhang, L. Tan, Angiotensin-(1-7) induces cerebral ischaemic tolerance by promoting brain angiogenesis in a Mas/eNOS-dependent pathway, *Br. J. Pharmacol.* 171 (2014) 4222–4232.
- [93] X. Xiao, C. Zhang, X. Ma, H. Miao, J. Wang, L. Liu, S. Chen, R. Zeng, Y. Chen, J. C. Bihl, Angiotensin-(1-7) counteracts angiotensin II-induced dysfunction in cerebral endothelial cells via modulating Nox2/ROS and PI3K/NO pathways, *Exp. Cell Res.* 336 (2015) 58–65.
- [94] J.C. Sullivan, P. Rodriguez-Miguel, M.A. Zimmerman, R.A. Harris, Differences in angiotensin (1-7) between men and women, *Am. J. Physiol. Heart Circ. Physiol.* 308 (2015) H1171–H1176.
- [95] A. Bukowska, L. Spiller, C. Wolke, U. Lendeckel, S. Weinert, J. Hoffmann, P. Bornfleth, I. Kutschka, A. Gardemann, B. Isermann, A. Goette, Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men, *Exp. Biol. Med.* 242 (2017) 1412–1423.
- [96] K.M.M. Colafella, K.M. Denton, Sex-specific differences in hypertension and associated cardiovascular disease, *Nat. Rev. Nephrol.* 14 (2018) 185–201.
- [97] K. Komukai, S. Mochizuki, M. Yoshimura, Gender and the renin-angiotensin-aldosterone system, *Fundam. Clin. Pharmacol.* 24 (2010) 687–698.
- [98] A. Mompeón, M. Lázaro-Franco, C. Bueno-Betf, D. Pérez-Cremades, X. Vidal-Gómez, E. Monsalve, M.M. Gironacci, C. Hermenegildo, S. Novella, Estradiol, acting through ERα, induces endothelial non-classic renin-angiotensin system increasing angiotensin 1-7 production, *Mol. Cell Endocrinol.* 422 (2016) 1–8.
- [99] F.P. Costa-Fraga, G.K. Gonçalves, F.P. Souza-Neto, A.M. Reis, L.A. Capettini, R. A. Santos, R.A. Fraga-Silva, N. Stergiopoulos, R.F. da Silva, Age-related changes in vascular responses to angiotensin-(1-7) in female mice, *J. Renin Angiotensin Aldosterone Syst.* 19 (2018), 1470320318789332, 1470320318789332.
- [100] A. Vargas-Castillo, S. Tobon-Cornejo, L. Del Valle-Mondragon, I. Torre-Villalvazo, A. Scholnik-Cabrera, M. Guevara-Cruz, E. Pichardo-Ontiveros, R. Fuentes-Romero, M. Bader, N. Alenina, A. Vidal-Puig, E. Hong, N. Torres, A.R. Tovar, Angiotensin-(1-7) induces beige fat thermogenesis through the Mas receptor, *Metabolism* 103 (2020), 154048.
- [101] L. Pasanen, H. Launonen, A. Siltari, R. Korpela, H. Vapaatalo, H. Salmenkari, et al., Age-related changes in the local intestinal renin-angiotensin system in normotensive and spontaneously hypertensive rats, *J. Physiol. Pharmacol.* 79 (2019) 199–208.
- [102] H.E. Yoon, E.N. Kim, M.Y. Kim, J.H. Lim, I.A. Jang, T.H. Ban, S.J. Shin, C.W. Park, Y.S. Chang, B.S. Choi, Age-associated changes in the vascular renin-angiotensin system in mice, *Ox. Med. Cell. Longev.* 2016 (2016) ID6731093–14.
- [103] M. Wang, J. Zhang, L.Q. Jiang, G. Spinetti, G. Pintus, R. Monticone, F.D. Kolodgie, R. Virmani, E.G. Lakatta, Proinflammatory proliferate within the grossly normal aged human aortic wall, *Hypertension* 50 (2007) 219–227.
- [104] S. Flavahan, F. Chang, N.A. Flavahan, Local renin-angiotensin system mediates endothelial dilator dysfunction in aging arteries, *Am. J. Physiol. Heart Circ. Physiol.* 311 (2016) H849–H854.
- [105] A. Siltari, R. Korpela, H. Vapaatalo, Bradykinin-induced vasodilatation: role of age, ACE1 inhibitory peptide, mas and bradykinin receptors, *Peptides* 85 (2016) 46–55.
- [106] M. Gupta, S.E. Thatcher, C.M. Boustany-Kari, R. Shoemaker, F. Yiannikouris, X. Zhang, M. Karounos, L.A. Cassis, Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice, *Arterioscler Thromb Vasc. Biol.* 32 (2012) 1392–1399.
- [107] F. Schinzari, M. Tesaro, A. Veneziani, N. Mores, N. Di Daniele, C. Cardillo, Favorable vascular actions of angiotensin-(1-7) in human obesity, *Hypertension* 71 (2018) 185–191.
- [108] P.D. Savage, J. Lovato, K.B. Brosnihan, A.A. Miller, W.J. Petty, Phase II trial of Angiotensin-(1-7) for the treatment of patients with metastatic sarcoma, *Sarcoma* 2016 (2016), 4592768.
- [109] K. Asish, R.R. Ghosh, K.E. Park, E.T.M. Mesut, L.D. Wilsbacher, D.E. Vaughan, A small molecule inhibitor of PAI-1 protects against doxorubicin-induced cellular senescence, *Oncotarget* 7 (2016) 72443–72457.