

Role of circulating factors in cardiac aging

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Abstract: Worldwide increase in life expectancy is a major contributor to the epidemic of chronic degenerative diseases. Aging, indeed, simultaneously affects multiple organ systems, and it has been hypothesized that systemic alterations in regulators of tissue physiology may regulate this process. Cardiac aging itself is a major risk factor for cardiovascular diseases and, because of the intimate relationship with the brain, may contribute to increase the risk of neurodegenerative disorders. Blood-borne factors may play a major role in this complex and still elusive process. A number of studies, mainly based on the revival of parabiosis, a surgical technique very popular during the 70s of the 20th century to study the effect of a shared circulation in two animals, have indeed shown the potential that humoral factors can control the aging process in different tissues. In this article we review the role of circulating factors in cardiovascular aging. A better understanding of these mechanisms may provide new insights in the aging process and provide novel therapeutic opportunities for chronic age-related disorders.

Keywords: Aging; parabiosis; cardiac aging; circulating factors

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Introduction

Aging is a pivotal contributor to several diseases and a major risk factor for numerous chronic conditions affecting the whole body (1). The aging process is responsible itself for several structural and functional changes and for the increased incidence of several cardiovascular risk factors such as hypertension, dyslipidemia and diabetes (2,3). The concepts that circulating factors may regulate the aging process have shed the light on this issue contributing to the development of novel therapeutic strategies to promote healthy aging (4-6).

The aim of this review is to retrace the potential role of circulating and soluble factors to halt, reverse or even

ameliorate the pathophysiological process of cardiac aging.

Aging, the magnitude of a problem

Aging may be considered a modern pandemic, associated with a serious social and economic impact. By 2050 more than two over nine billions of estimated people will be older than 60 years and yet in 2017 there will be more people over 65 than under 5 years old (7). In the European Union (EU), by 2060, approximately one third of the population will be aged 65 or over (Commission, 2012 #3816) whereas in the United States, 1 every 7 Americans is already older than 65 (8).

The progressive increase in life expectancy is associated

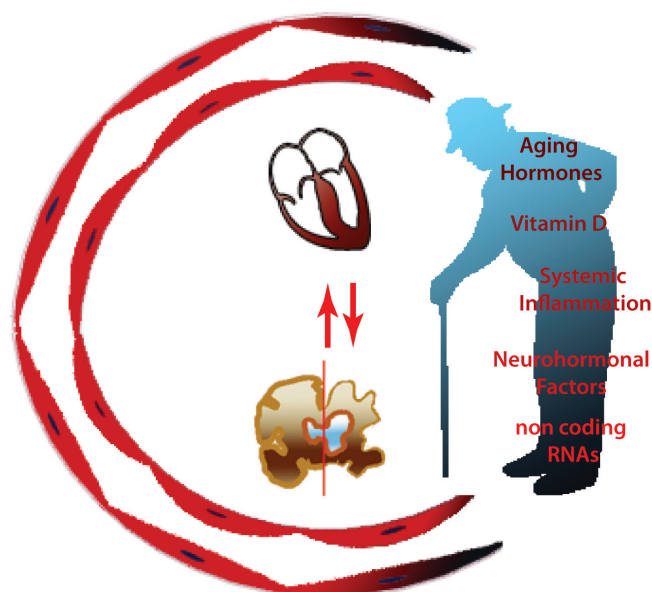


Figure 1 Aging is characterized by systemic changes that lead to a progressive decline in tissue function. Circulating factors contribute to activate molecular pathways that regulate cardiac aging. The profound interconnection between heart and brain becomes more evident with aging and thus understanding the specific role of these factors may represent a novel therapeutic opportunity for chronic disorders.

with higher prevalence of chronic age-related disease (9). In this light, understanding the mechanisms underlying this process and the changes that physiologically occur with time, is pivotal to improve the quality of life of the elderly and reduce the burden of age-related diseases (10).

Aging is considered an invariable and progressive time-dependent decline in organism functions due to the constant gait of time (11) and is associated with deleterious changes leading to functional impairments typical of the elderly (12).

Aging is itself a major risk factor for several diseases, mostly chronic. Cardiovascular disease, cancer, degenerative disorders, immune-mediated diseases show indeed a higher prevalence in the elderly population, mainly because of the loss of the adaptive response to insults and an imbalance in tissue homeostasis (13). The aging process undeniably affects the cardiovascular system and the prevalence of cardiovascular diseases increases overtime. As early as in the 1970s, McKee and coll. identified in the Framingham study that older age was an independent risk factor for heart failure (HF) and aging was a predictor of worse

prognosis in those patients (14). Aging indeed contributes significantly to the global burden of cardiovascular risk factors, HF, cardiomyopathies and ischemic heart disease (2). In particular, HF represents a major clinical and socio-economical problem characterized by significant morbidity and mortality and progressive increase in healthcare expenses, especially in those older than 65 years (15,16).

The aging process induces structural and functional changes such as vascular stiffening, myocyte hypertrophy and increased wall thickness, increased myocardial fibrosis and extracellular matrix (ECM) remodeling leading together to diastolic dysfunction characterized by reduced active filling of the left ventricle. These changes may explain the higher prevalence of heart failure with preserved ejection fraction (HFpEF) in the aging population (17-19). While aging may not represent the direct cause of HF, this process is associated with a lower threshold for clinical signs and symptoms of the disease (15,20).

Patients with HFpEF present normal ventricular volumes and ejection fraction, as opposed to heart failure with reduced ejection fraction (HFrEF), and a severe impairment in ventricular relaxation (21,22). Furthermore, those patients are often female and usually older with numerous comorbidities such as obesity, diabetes, and hypertension when compared to patients with HFrEF (23).

Despite several efforts, to date there are no specific treatments for this condition, therefore it appears crucial to develop novel strategies to understand the pathophysiological background and the possible therapeutic targets for patients with HFpEF (24).

Circulating factors in cardiac aging

A number of recent evidences indicate a pivotal role for circulating factors associated with aging in affecting the function of the cardiovascular system (*Figure 1*). While until recently the concept that circulating factors may affect cardiovascular health was limited mainly to lipids (25) and systemic factors mainly represented useful biomarkers for the diagnosis and for risk stratification of individuals at higher risk of cardiovascular disease (26-29), their role in promoting aging and pathological process has been recently highlighted (30-33).

Blood, circulating factors and *in vivo* parabiosis

The concept that blood carries either beneficial or detrimental factors is not a novel finding. Hippocrates

from Kos identified the four “evil humors” responsible for several disease and proposed the bloodletting, known as phlebotomy, as effective treatment for those conditions (34,35). In most recent times, bloodletting was considered still an available option for untreatable disease (36) and Sir William Osler recommended the phlebotomy as a therapeutic choice in his textbook “*The Principles and Practice of Medicine*” (37,38).

Blood carries circulating factors, like hormones, which can affect organs distant from their site of production. The diversified world of the hormones has not yet been completely characterized and a number of circulating factors with this role have yet to be identified (39). While circulating factors are important biomarkers in different conditions, growing evidences indicate their role as a therapeutic option for cardiovascular disease (40,41), liver disease (40), cancer (42), and neurological disease (43).

To study the possibility that a shared circulatory system may affect a specific condition, Paul Bert, in the mid-1800s, introduced the surgical technique of parabiosis in which, he hypothesized, surgically connected animals may develop a shared circulation (44). Animals joined in parabiosis develop a shared blood circulation, with rapid and continuous exchange of cells and soluble factors at physiological levels through their common circulatory system (45). This surgical technique has been underemployed until the 1970s when, Coleman and Coll., conjoined obese mice and diabetic mice in parabiosis with WT mice, paving the way to discover the role of leptin and leptin receptor in diabetes (46,47). More recently, in a revival of this interesting old-fashioned technique, heterochronic parabiosis, a specific experimental procedure whereby two animals of different ages are joined together, allowed to study the role of shared circulation in several conditions, such as muscular atrophy, neurodegenerative disorders, cardiovascular diseases, and to discover several soluble factors that can affect specific phenotypes in experimental animals (48-52).

Growth differentiation factor 11 (GDF11) was identified as the factor that recapitulates the effect of heterochronic parabiosis on cardiac muscle, reversing age-related cardiac hypertrophy (50). Further studies have extended the effect of GDF11 to restoration of skeletal muscle function and improved angiogenesis in the brains of aging mice (49,53).

Other studies have challenged these results, in part because of the homology with myostatin (GDF8), that render difficult to discriminate the effect of the specific proteins (54,55). Some of these discrepancies may be explained by the complexity and redundancy of the

pathways and by the role of specific post-translational forms of GDF11, including specific antagonists (56,57). GDF11 has shown a dose-dependent effect on cardiac mass (58) and, although further studies are needed to clarify its specific role, the possibility of targeting specifically cardiomyocytes and other cells with aging hormones may represent a novel therapeutic option.

Circulating factors in aging: the intersection between brain and heart

Heart and brain have an intimate relationship, more evident in aging when the link between heart dysfunction and the brain activity become manifest (59).

Cardiovascular pathologies, cerebrovascular disorders and neurodegenerative diseases are prominent features of aging and dementia and cognitive impairment represent the main cause of disability in older people. The most common forms of dementia, Alzheimer’s disease (AD) and vascular dementia (VaD), cover about 80-90% of all dementias (59) and these pathologies are usually associated with risk factors for cardiovascular disorders and heart dysfunction.

Traditionally VaD, a pathological condition due to embolic stroke, cardiac dysfunction or age-related vascular stiffening responsible for chronic hypoperfusion, was formerly considered the entity promoted by diabetes mellitus, hypertension, hypercholesterolemia, obesity or closely related to cardiac pathologies. Recently, a large body of evidence shows that cardiovascular risk factors are also associated with AD, considered a purely neurodegenerative disorder (60).

In normal brain aging and in AD pathology, two neuropathological hallmarks characterize the brain tissue: extracellular deposits of amyloid beta (A β) protein in which misfolded A β fibrils are organized in senile plaques and intraneuronal aggregates of hyperphosphorylated and misfolded tau protein that become extraneuronal (“ghost” tangles) when tangle-bearing neurons die (61). A β protein spontaneously self-aggregates into other multiple physical forms than fibrils and one of them consists of oligomers. Most evidences support the notion that the early stages of A β oligomerization rather than the fibrils in senile plaques are responsible for the toxic effects of A β at synaptic level (62). During aging and in the progression of AD, synaptic plasticity, capacity of sprouting and neuronal integrity is compromised. Recent studies suggest that the direct abnormal accumulation of A β oligomers in the neuronal terminals might contribute to the synaptic damage and

plasticity deficit, leading to cognitive impairment although the biological metastable nature of A β oligomers makes difficult their synaptic detection (62).

In addition to A β and Tau effects, multiple and complex factors have been identified in AD pathogenesis including oxidative stress, mitochondrial damage, inflammatory responses and changes in cellular communication. This complexity is the main reason by which there is still a lack of understanding of initiating disease mechanisms and the absence of effective treatment options (63). A large body of evidence shows that caloric restriction, exercise, mental activity and the control of cardiovascular risk factors can counteract the aging brain (52).

Recent findings have highlighted the importance of circulating factors in determining and reversing the course of brain aging and the profound intersection between heart and brain (49,51,52). The exposure of old animals to young blood in the later phase of their life is able to rejuvenate synaptic plasticity and to improve cognitive function therefore counterbalancing the aging process (52). Furthermore, in *Xenopus* oocytes, macrophage-derived soluble factors may directly activate N-methyl-d-aspartate (NMDA) receptor subtype NR1a/NR2B delaying the onset of AD (43). The exposure to young blood enhanced the complex interplay between vascular system and cognitive function. Indeed, circulating factors in young animals improved vascular function in the aging mouse brain, leading to increased blood flow and ultimately to increased neural activity and functioning (49).

Taken together these notions suggest that blood-borne factors are essential to ameliorate the brain aging phenotype (51,64).

Although these experiments have been performed in rodents, these results have sufficiently convinced the researchers to initiate a human study, testing the transfusion of young human plasma in patients with different types of dementia. The study (Plasma Study), started in September 2014 with final data collection in November 2016, enrolled 18 demented subjects and the preliminary data are not yet known (65) (<http://clinicaltrials.gov/ct2/show/NCT02256306>). More recently a pay-to-participate trial (Ambrosia's trial) started in Monterey (CA) (66). Healthy volunteers and not necessarily elderly—the trial is open to anyone 35 and older—are enrolled in order to receive transfusion of plasma from donors under age 25 to test for more than 100 biomarkers that may vary with age. For the study characteristics, the trial is giving rise to huge ethical problems.

Systemic inflammation

Inflammation is the common pathway occurring within the vasculature and throughout the body in response to an injury (67). Systemic inflammation associated with aging, even in absence of a specific pathological process (68), has a key role in determining structural and functional changes in the aging myocardium (13), underlying the pathophysiological process of cardiac frailty and cardiovascular pathology (69). Even in the absence of chronic conditions, circulating inflammatory factors, such as interleukin (IL)-6, tumor necrosis factor (TNF)- α and soluble TNF receptor-1 (TNFR-1), and C-reactive protein (CRP) are usually two to four folds higher in the elderly compared to young subjects (70). The inflammatory response is tightly associated with increased production of reactive oxygen species (ROS), mitochondrial damage, and accelerated senescence (71). Furthermore, subtle inflammatory processes lead to arterial stiffening and endothelial dysfunction resulting in age-related inflammatory chronic disease, i.e., atherosclerosis and hypertension (69,72,73). Hosford-Donovan and Coll. demonstrated that higher level of CRP were independently associated with hypertensive phenotype in 65–70 years old women, postulating that chronic inflammation may influence blood pressure's regulation in the elderly leading to increased vascular stiffening and accelerated atherosclerosis (74).

IL-6 levels are a marker of cardiovascular disease and serum concentration of this cytokine increases with age (13,75). Elevated levels of IL-6 are found in aged mice hearts and deletion of myocardial Insulin-like Growth Factor (IGF)-1 Receptor rescues the aging cardiac phenotype suggesting a possible link between these two factors (76). In humans, elevated level of IL-6 were associated with higher cardiovascular mortality over a period of 3 years of follow-up indicating that systemic inflammation may contribute to the cardiac aging process (77).

The measurement of serum concentration of CRP is a well-known useful marker for patients at higher risk of atherosclerotic disease and the prognostic value of this evaluation is comparable to blood cholesterol measurements (78,79). Furthermore, CRP concentration increases significantly with age, without gender differences, and provides a reliable measurement in assessing the risk of future cardiovascular events (80,81). Although CRP can be considered as a useful marker for cardiovascular disease, it is unlikely that specific treatments targeting this molecule may provide a net clinical benefit (82).

While several attempts to target this condition using therapeutic anti-inflammatory regimens to reduce cardiovascular mortality have failed (83), targeting the whole CRP/IL-6/IL-1 axis with monoclonal antibodies could open new therapeutic lines in cardiovascular pathology and offer novel insights for the aging process of the heart (82). An interesting finding indicates an inverse relationship between serum level of testosterone and CRP, warning regarding the increased risk of cardiovascular disease in aging men and linking the neurohormonal axis to the inflammatory process (84).

While targeting systemic inflammation may represent an interesting therapeutic option, so far little evidence are available indicating its employment in the near future (83).

Vitamin D

Vitamin D deficiency is a serious health issue worldwide, requiring special attention (85-87). Vitamin D is a fat-soluble vitamin responsible for calcium and phosphorus homeostasis (88,89). Vitamin D deficiency has been previously associated with poorer neuropsychological function, metabolic syndrome, arrhythmias and coronary artery disease, and shares a complex interplay with other cardiovascular risk factors (88-93). Numerous studies hypothesized that low levels of Vitamin D are inversely associated with cardiovascular disease and cardiac aging, however evidences and data from large clinical trials are limited (94-96). By contrast, higher levels of Vitamin D have been associated with longer leukocyte telomere length (LTL) suggesting a potential beneficial effect of this vitamin on the aging process (97). Therefore, besides the well-known effect of sun exposure on bone's health, preservation of normal values of Vitamin D appears beneficial for cardiovascular health and healthy aging process (85,94).

Neurohormonal factors

Sympathetic nerve stimulation (SNS), Renin Angiotensin Aldosterone System (RAAS) and Natriuretic Peptides (NP), are part of the defense system adopted by the body to maintain fluid homeostasis and vascular resistance and provide proper perfusion to distant organs (98). Aging is associated to increased activation of the neurohormonal system, in part as a result of an imbalance between production and clearance of vasoactive molecules (99) and can in part explain the high incidence and prevalence of HF

in the elderly (15). While the activation of these systems during the early phase of HF may normalize cardiac output and perfusion, chronic activation has a deleterious impact on the outcome of this condition, promoting structural and functional changes in the myocardium and the vasculature that eventually contribute to decompensated HF (100-103). Thus, in order to prevent the progression of HF and to improve morbidity and mortality, several treatments targeting this axis and modulate their activity have been developed (104,105). HF is also a risk factor for neurodegenerative disorders, possibly because impaired blood flow and neurohormonal activation favor accumulation of A β plaques and neurofibrillary tangles (106), indicating the strict link between aging and chronic disorders.

Activation of RAAS, which is pivotal for fluid and blood pressure homeostasis (98), leads to increased level of Ang-II, which has a pro-hypertrophic and pro-fibrotic effect. High levels of Ang-II are tightly associated with cardiomyocyte hypertrophy, ECM remodeling and increased collagen deposition and ultimately to cardiac fibrosis (13,107,108). These changes at the tissue level contribute to clinical manifestations of cardiac aging, such as diastolic dysfunction, impaired relaxation and reduced compliance of the ventricle, and promote the progression to heart failure with ejection fraction either preserved (HFpEF) or heart failure with ejection fraction either reduced (HFrEF), both in humans and in animals (109). Recent studies in hypertensive and aging animals have shown how hyperactivation of the RAAS system reduces ventricular compliance through increased titin-based myocardial stiffness, ultimately contributing to diastolic dysfunction and development of HFpEF, underlining the pleiotropic negative effects of the persistent increased activation of RAAS (110,111). Persistent activation of RAAS also stimulates β -amiloid production (112), an important link between cardiac and neuronal aging (113). Ang-II has important pro-inflammatory effects, leading to increased secretion of TNF α , IL-1 β , and IL-6 (114). With aging, increased levels of Ang-II stimulate NADPH oxidase 4 (NOX4) on the mitochondrial membrane enhancing the oxidative damage, a cornerstone of the aging process (115-117). Ang-II enhances cardiac fibroblast proliferation through NOX4/ROS-dependent IL-18 induction, MMP9 and p38 MAPK activation, promoting cardiac remodeling and diastolic dysfunction (118,119), while blockade of the RAAS delays the fibrotic response of the myocardium (120).

In humans, proteins of the RAAS system can be found in the urine of healthy aged individuals (121) suggesting

an increased activity of this system, possibly explaining the progressive cardiac and kidney failure (122-124), the increased incidence of hypertension, due endothelial dysfunction and arterial stiffness (125), the high incidence of metabolic syndrome and diabetes (126), and the aging phenotype (127,128).

RAAS blockade with angiotensin converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs) is able to reduce cardiovascular mortality independently from blood pressure lowering, either in humans or in animals (129-131), ameliorates cardiac hypertrophy and myocardial fibrosis (132), endothelial dysfunction and arterial stiffness (133) and improves animal survival (134). Furthermore, treatment with ACE-I and ARBs represent a valid therapeutic option for aged people (105,135,136), improving cerebral blood flow and reducing the 1-year risk of fall in elderly people (137), even though in hypertensive patients it is associated with worse gait performance (138) and appears to have no effect on walking distance or on age-related decline of muscle strength (139).

Aging is also associated with elevated levels of endothelin-1 (ET-1), in part stimulated by Ang-II (140), promoting the development of the an aging phenotype characterized by ECM remodeling, increased collagen deposition and endothelial dysfunction contributing to a progressive impairment of the diastolic function and eventually to HFpEF (141,142). ET-1 stimulates collagen deposition through both endothelin receptor type A (ETA) and type B (ETB), and administration of ETA receptor antagonists is able to reduce either cardiac or renal fibrosis (141,143,144). Furthermore, antidepressant treatment with venlafaxine, commonly used in elderly population, is able to modulate TGF- β expression and to reduce brain damage in a rat model of ET-1 induced stroke, confirming the key role of ET-1 in the progression of vascular damage (145).

NP are a family of different peptides acting directly on cardiovascular and renal systems, balancing fluid homeostasis (146) and promoting vasorelaxation, natriuresis and diuresis (147). Plasma levels of NPs are crucial in the evaluation of patients with HF (146,148). Elevated serum levels of brain natriuretic peptide (BNP) are independently associated with poorer outcomes (149,150) and predict higher in-hospital mortality in very elderly patients admitted for HF (151). BNP levels are elevated in the aging population compared to the younger counterparts even in the absence of a clear diagnosis of HF (152,153). BNP levels are persistently elevated in the elderly independently from blood pressure changes, renal function, atrial volumes,

myocardial mass or other age-related changes (154,155) suggesting the importance of these circulating factors in normal aging. NPs increase the production of cGMP (156) and are degraded mainly through the enzyme Neprilysin (NEP), a zinc-dependent enzyme widely expressed throughout the body (157).

In animals, increased levels of NPs or chronic inhibition of NEP are able to prevent the progression of cardiac aging (158,159). Similarly, in humans, simultaneous inhibition of NEP and Ang-II type-1 receptor (AT1R) with LCZ-696 (which is composed by Valsartan and Sacubitril) has shown to reduce significantly mortality and hospitalization in patients with HF (160). It is estimated that this compound, recommended in the most recent guidelines on treatment of HF (135,136), may add 1 to 2 years to life expectancy in these patients (161).

Organism homeostasis and the correct balancing of circulation factors are essential for physiological and healthy aging. Imbalance of these axis significantly affects the aging phenotype, thus therapeutic strategies acting on these targets may ameliorate lifespan and healthspan of the aging population.

MicroRNAs (miRNAs) and long non coding RNAs (lncRNA)

Circulating miRNAs are single-stranded and non-coding RNA molecules of approximately 22 nucleotides regulating several biological activities (162). miRNAs emerged as important biomarkers and therapeutic target for several conditions, including HF (163). Numerous miRNAs, such as miR-146, miR-155, miR-21, miR-126 appear to be involved in the aging process (164,165), in cardiac remodeling observed with aging (166,167) and are associated with prognosis and response to therapy in HF (163).

The miR-34 family, which includes miR-34a, miR-34b and miR-34c and is important in cancer formation, metastasis, and cell viability (168), is tightly associated with the aging process (169). Indeed, elevated levels of miR-34 are found in the heart of old mice (170) and the inhibition of its downstream target, the protein phosphatase-1 regulatory subunit-10 (PNUTS) is associated with increased cardiomyocyte apoptosis, telomere attrition and cardiac contractile impairment, typical hallmarks of cardiac aging (171).

Like miRNA, lncRNA, which are the vast majority of non-coding RNAs and are longer than 200 nucleotides, are

also important modulator of the aging process (172,173). lncRNAs are key regulator of the aging process and play an important role in the onset and progression of cardiac aging (174,175). Senescence-associated lncRNAs (SAL-RNAs) may indeed influence the aging phenotype, regulating cardiac function and myocardial fibrosis (176). In particular, SAL-RNA1 appears to delay cardiac aging (176) while SAL-RNA2 and SAL-RNA3 promote the survival of the senescent fibroblasts through the increased expression of p53 (174). Furthermore, the lncRNA H19 is an important modulator of the aging process, negatively affecting cell proliferation and promoting cellular senescence (177,178).

miRNAs and lncRNAs, interesting and useful biomarkers, represent also a potential therapeutic target to modulate the aging process. Recently, Kaneko and Coll. demonstrated that treatment with ARBs reduces the serum level of miR-146a, miR-149, miR-150, and miR-342-3p, improving survival rate and ameliorating congestion of the animals treated, suggesting possible crossroads between these two pathways of cardiac aging (179). Further studies are needed to elucidate the mechanism underlying this process and to develop tailored therapy to prevent the progression of the aging phenotype.

Conclusions

The aging epidemic that we are observing will benefit from our understanding of the molecular mechanisms that regulate cardiovascular aging with clear repercussions on other interconnected systems. The recent notions that circulating factors may contribute to control aging and the chronic illnesses that are strictly connected to this phenotype can be seen as an intriguing finding but also as a new therapeutic opportunity. A number of therapeutic options that interfere with circulating mediators of cardiac aging have shown a clear role in ameliorating the burden of chronic cardiovascular conditions. Unraveling the complex systemic mechanisms that regulate cardiac aging will provide novel pharmacologic strategies with a clear impact on quality of life in a progressively aging world.

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