Role of hydrogen sulfide in cardiovascular ageing

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Abstract

Cardiovascular diseases are the main cause of morbidity and mortality in the Western society and ageing is a relevant non-modifiable risk factor. Morphological and functional alterations at endothelial level represent first events of ageing, inevitably followed by vascular dysfunction and consequent atherosclerosis that deeply influences cardiovascular health. Indeed, myocardial hypertrophy and fibrosis typically occur and contribute to compromise overall cardiac output. As regards the intracellular molecular mechanisms involved in the cardiovascular ageing, an intricate network is emerging, revealing a role for many mediators, including SIRT1/AMPK/PCG1α pathway, anti-oxidants factors (i.e. Nrf-2 and FOXOs) and pro-inflammatory cytokines. Thus, the search for pharmacological and non-pharmacological strategies that can promote a "healthy ageing", in order to slow down age-related machinery, are currently an exciting challenge for the biomedical research. Interestingly, hydrogen sulfide (H_2S) has been recently recognized as a new player capable to influence intracellular machinery involved in ageing and then it is view as a potential target for preventing cardiovascular diseases. Therefore, this review is focused on the role of H_2S in cardiovascular ageing, and on the evidence of the relationship between progressive decline in endogenous H_2S levels and the onset of various cardiovascular age-related diseases.

Keywords: Ageing; Cardiovascular diseases; Hydrogen sulfide; Aged-related intracellular pathways

1 Introduction

Cardiovascular diseases (CVDs) are the main cause of morbidity and mortality in the Western society and ageing is the primary non-modifiable risk factor for CVDs, in fact about 80 % of cardiovascular deaths occur in over-65

patients [1,2].

In 1939, for the first time, McCay and colleagues observed that a caloric intake restriction in rodents increased their lifespan, demonstrating that it was possible to influence the evolution of this inevitable physiological process [3]. Later, this evidence has been also confirmed in primates [4]. Therefore, many pharmacological efforts are presently focused on slowing down the ageing process in order to reduce age-related diseases (mainly the CVDs), identifying new effective approaches. In this context, hydrogen sulfide (H_2S), the third endogenous gasotransmitter, recently emerged as new player capable to influence intracellular machinery involved in ageing and then it is view as a potential target for preventing CVDs.

2 Impact of ageing on cardiovascular system

Alterations in endothelial structure and function are the first events occurring during ageing, as a consequence of oxidative stress and inflammation, inevitably followed by vascular dysfunction, which deeply influences cardiovascular health [5] (Fig. 1).

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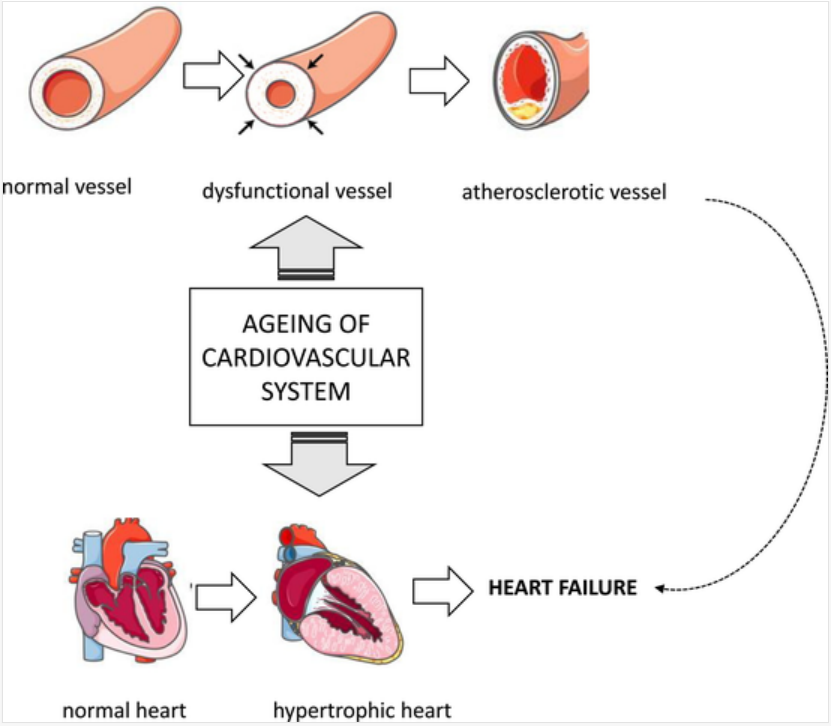
Fig. 1



Ageing-related events occurring at cardiovascular level.
The reduction of the availability of nitric oxide and alterations of homeostasis of ions flow through ion channels trigger the endothelial dysfunction, which negatively affects dilation of vasculature and promotes its stiffness. Progressive thickening and hyperplasia cause alteration in the morphology of vessels and then exacerbating the functional alterations. Atherosclerosis is a main consequence of the age-related vascular remodeling. Beside the effects on the vascular system, ageing has remarkable effects on the heart. Myocardial hypertrophy, characterized by an increase of left ventricular (LV) mass, is a common feature of LV remodeling and associated with worsening the overall cardiac performance. Heart failure is one of the most common morbidity of elderly.

The reduction in endothelial nitric oxide synthase (eNOS) activity and a lower availability of nitric oxide (NO) are main aspects of endothelial dysfunction [6,7]. NO is well-known as a critical vasodilator produced by endothelial cells, regulating vascular tone, but also inhibiting vascular inflammation and thrombotic events. Thus

a reduced production of NO is associated with an impaired control of vascular tone [8,9]. In addition, reduced density of voltage-dependent and big conductance calcium-activated potassium channels (Kv and BK, respectively) has been described in aged coronary arteries [10,11]. Since potassium channels, particularly BK, are recognized as regulators of vascular tone through NO-pathway, this alteration further worsens the impaired vasodilation.

Therefore, endothelium dysfunction negatively affects dilation of vasculature and promotes its stiffness [12–14]. Moreover, endothelium becomes more porous and vascular smooth muscle (VSM) cells migrate into sub-endothelial spaces and deposit extracellular matrix proteins resulting in intimal thickening. Progressive intimal thickening is generally due to enhanced elastin degradation and collagen deposition in the vascular media as well as intimal hyperplasia, causing alteration in the morphology of vessels and then exacerbating the functional alterations [15].

Notably, the age-related vascular remodeling is a prodromal event to atherosclerosis [16]. This latter is one of the most important disease involving vessel wall and is considered as a key risk factor in the development of CVDs, including hypertension, stroke and coronaropathies [17] (Fig. 1).

The decline of endothelial function is also strongly linked to the pathogenesis of atherosclerosis; low density lipoprotein (LDL) cholesterol can be oxidized in the initial stage of atherogenesis, leading to the activation of endothelium and initiation of immune system response, which feeds further oxidative stress and inflammation and then the progression of atherosclerosis [18]. In fact, inflammation is the third key element in the relationship between lipids and endothelium. Pro-inflammatory cytokines contribute to increase atherosclerotic region, through arterial plaque formation and additional cell apoptosis, leading to lipids expulsion into adjacent plaque areas [17]. The formation of the atherosclerotic plaque represents a source of vulnerability because it leads to the formation of thrombi which are the main cause of myocardial infarction or stroke [19].

In this regard, recently atherosclerosis-prone mice, transfected with the longevity-associated variant (LAV) of Bactericidal/Permeability-Increasing Fold-Containing Family B member 4 (BPIFB4) protein (recognized as a powerful boost for endothelial vasorelaxation and revascularization), showed reduced endothelial dysfunction and slower atherogenic plaque progression compared with wild-type controls. Moreover, the LAV-BPIFB4 treatment of arteries explanted from atherosclerotic patients highlighted an anti-inflammatory profile together with release of the atheroprotective IL-33 [20], confirming the closely correlation among these events.

Worthy of note, in 2020, Tyrell and colleagues suggested that atherogenic events occurred even before a condition of hypercholestoremia. They demonstrated that ageing significantly elevated IL-6 levels and impaired mitochondrial function in vessels, by enhancing mitophagy. Therefore, these recent results suggest that independently from high levels of lipids, impairment of vascular mitochondrial bioenergetics and increased inflammation are responsible for the onset of atherosclerotic changes [2]. Likewise, Bennet's group previously observed in apolipoprotein-E knockout (apoE$^{-/-}$) mice that atherogenesis was promoted through mtDNA damage-induced mitochondrial dysfunction, before the plaque formation [21]. Taken together, these results suggest that atherogenesis started before the evidence of morphological modifications.

Beside the effects on the vascular system, ageing has remarkable effects on the heart [22] (Fig. 1). Clinically, it has been widely reported that heart weight increases whereas the number of cardiomyocytes is reduced, together with a parallel decline in regenerative capabilities and cardiac output [23]. As well, compensatory mechanisms, such as fibroblast proliferation, are recruited to transitorily improve myocardial response in order to maintain a sufficient ejection fraction [24–26]. Myocardial hypertrophy, characterized by an increase of left ventricular (LV)

mass, is a common feature of LV remodeling and is recognized as an efficient short-term mechanism, even if over a long term it is detrimental, worsening the overall cardiac performance.

Accordingly, aged mice (20–22 months of age) exhibit increased LV weight and cardiomyocyte volume and reduced cardiomyocytes number. As well, they show reduced ventricular function, confirming the pathological role of cardiomyocyte senescence in the aged heart. Also in human, hypertrophy, altered LV diastolic function and reduced LV systolic capacity, impaired endothelial function and increased coronary arterial stiffness are frequently described in elderlies and cardiac fibrosis is the final manifestation.

Heart failure is one of the most common morbidity of elderly, with an overall prevalence of about 10 % in the over-80 people [27]. In humans, end-stage heart failure is characterized by elevated levels of the senescence marker p53 in myocardium. Accordingly, in patients with hypertrophic cardiomyopathy higher p53 expression was reported, when compared to non-failing heart. Conversely, pharmacological interventions for heart failure, such as neprilysin inhibition, reduced circulating levels of this senescence molecule [28–30]. Together with the mechanical consequences, cardiac fibrosis can slow down even the electric impulse propagation and influence heart rate, suggesting that ageing can also promote cardiac arrhythmias [31].

3 Mechanisms underlying ageing processes

In this scenario, an intricate network among the recognized players is emerging, therefore a comprehension of molecular pathways involved in the ageing is necessary in order to plan an effective intervention focused on ageing-related CVDs (Fig. 2).

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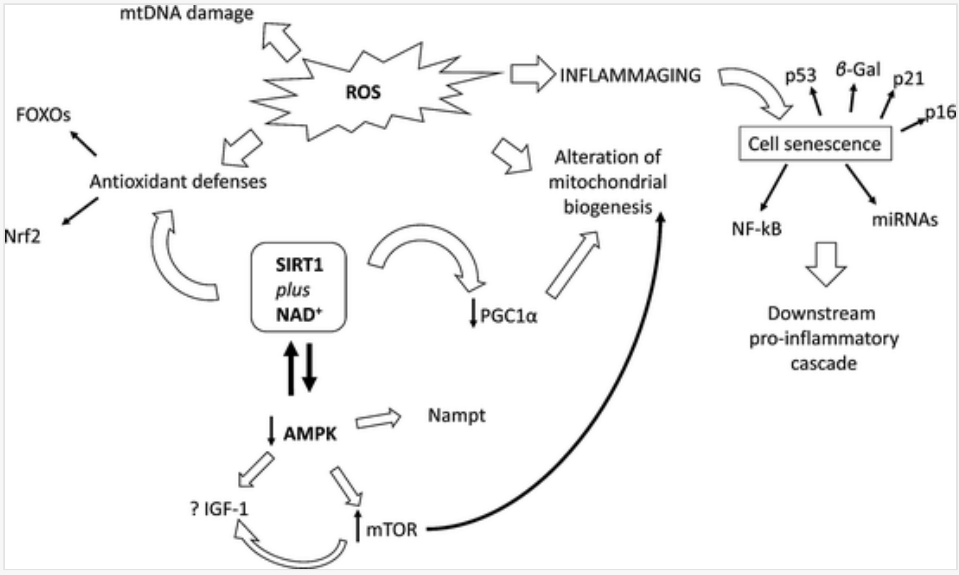
Fig. 2



Intracellular mechanisms engaged during the ageing.
Reactive oxygen species (ROS) play central role in senescence process and are responsible for mtDNA damage, impairment of antioxidant defenses and alteration of mitochondrial biogenesis. Besides, cell senescence, characterized by a chronic low-grade secretion of pro-inflammatory molecules (defined as inflammaging), has been described. Several markers of cell senescence have been recognized, including β-Gal, p53, p16, p21 and NF-kB and IL6. Mitochondrial biogenesis and antioxidant defenses may be significantly influenced by SIRT1 activity. SIRT1 and AMPK are closely linked and orchestrate the whole ageing-related machinery, regulating other players such as mTOR.

3.1 Free-radical theory and mitochondrial biogenesis

The key role of reactive oxygen species (ROS) and the central role of mitochondria in senescence processes are well-established. Indeed, the “free radical theory” (conceived in 1950s) demonstrated that, mitochondria produce free radical molecules (such as ROS) as a byproduct of cellular respiration; these species can promote adaptive responses, contributing to stress resistance and longevity. Nevertheless, when high ROS levels are produced, beyond the threshold of buffering capacity, they induce mitochondrial dysfunction, triggering the genetic damage, including mutations in nuclear and mitochondrial genomes [32], epigenetic changes, and impairment of mitochondrial biogenesis. In mitochondrial biogenesis, i.e. balancing between growth and division of organelles, peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) is a critical endogenous modulator [33,34].

Notably, PGC-1α-knockout mice are associated with increased oxidative stress, mitochondrial abnormalities, reduced telomerase activity, resulting in increased senescence markers at vascular level [35].

Mitochondrial channelopathies have been also described in ageing. Indeed, potassium, calcium and anion flows are affected and homeostasis is altered. In heart of 24-month-old rats a depolarized mitochondrial membrane potential has been found and, similarly to sarcolemmatic, a reduced density of mitochondrial potassium channels (mitoKs) has been shown, with heavy repercussions on susceptibility to damaging events, in particular myocardial tolerance against ischemia/reperfusion (I/R) is reduced in ageing and increased injury in rodents and human has been reported [36].

3.2 Cell senescence and chronic inflammation

Oxidative stress, mitochondrial dysfunction and DNA damage contribute to cell senescence, characterized by a stop proliferating and secretion of pro-inflammatory molecules (known as the senescence-associated secretory phenotype, SASP), that causes chronic sterile inflammation and tissue remodeling. Worth of note, in 2000, Franceschi defined the typical condition of low-grade systemic inflammation associated to senescence as “inflamm-ageing” [37], in which high levels of pro-inflammatory cytokines are produced. Indeed, the production of pro-inflammatory IL-6 rises in ageing. In contrast, high levels of anti-inflammatory cytokines have been detected in blood samples of centenaries [38].

In senescent cells, high levels of lysosomal β-galactosidase activity (SA-βgal) have been also measured and it is recognized as a reliable marker of senescence, as well as p16, p21 and IL-6 [39]. Senescent cells in endothelium and cardiac tissue have been associated with hardening of the heart muscle and stiffening of the vascular wall, resulting in angina, dyspnea and heart failure; whereas endothelial cell senescence has been found in dysfunctional vessels [40,41].

In this context, nuclear factor kappa-B (NF-κB) is probably the most important transcription factor responsible for regulating gene expression of factors that control cell adhesion, proliferation, inflammation, redox state and tissue-specific enzymes [42,43]. At cardiovascular level, dysregulated NF-κB is relevant, since it impairs endothelium-dependent dilatation and induces myocardial inflammation and fibrosis [44,45].

3.3 SIRT-1/ AMPK pathway

Strong scientific evidence suggests that sirtuin1 (SIRT1)/AMP-activated protein kinase (AMPK) pathway is a promising anti-ageing target, which drives the expression of cytoprotective and antioxidative genes and modulates cell metabolism.

In particular, sirtuins are deacetylase enzymes deeply involved in cell metabolism and thus in the control of the response to caloric restriction, a well-described model to extend lifespan. Though seven isoforms of sirtuin have been described, the isoform SIRT1 is defined as the protein of longevity [46]. Noteworthy, silencing of gene Sir2 –encoding for SIRT1 – significantly shorted lifespan, in yeasts, nematodes and also in mammals [46].

It has been demonstrated that SIRT1 protects the heart from I/R injury, hypertrophy and cardiomyocytes apoptosis [47]. Accordingly, SIRT1 over-expression leads to reduced myocardial hypertrophy, interstitial fibrosis, oxidative stress and senescent markers, improving the cardiac function [48]. Moreover, pharmacological activation of SIRT1 can ameliorate the endothelium function, activating eNOS and preventing atherogenesis [49].

Conversely, excessive levels of ROS may be responsible for the decreased SIRT1 activity in ageing, which facilitates the senescent-like phenotype (detected by specific markers, including p53 and p21) [50].

Evidence acquired by *in vitro* and *in vivo* studies shows that SIRT1 regulates AMPK, p53, mammalian target of rapamycin (mTOR), NF-κB, Forkhead transcription factor (FOXO) family and PGC-1α, by direct deacetylation [51–53].

In particular, SIRT1 removes, in the presence of cofactor NAD^+, acetyl groups from histone and non-histone proteins, regulating the transcription factors, such as nuclear factor erythroid 2-related factor (Nrf-2) and FOXO family, involved in the antioxidant defenses. Recently, a key role has been suggested for NAD^+. Indeed, conditions that increase lifespan, such as caloric restriction or exercise, improve the availability of the cofactor [54]. In preclinical models, it has been demonstrated that NAD^+ availability decreases with age in several tissues and that supplemental NAD^+ precursors can ameliorate many age-related cellular impairments [55].

Nrf-2 regulates the transcription of numerous antioxidant genes, preserving the cellular homeostasis and tuning the biological response to oxidative stress. Therefore, Nrf-2 has been indicated as “the master regulator of antioxidant responses” [56–58].

In physiological conditions, Nrf-2 is restrained into the cytoplasm and is bound in an inactive complex with Kelch-like ECH-associated protein 1 (Keap1) dimer. A disruption of the intracellular redox status, due to high ROS levels, causes Keap1 dimer division by the breaking of disulfide bonds between cysteine residues. This event promotes the release of Nrf-2 that, after its phosphorylation, translocates into the nucleus and increases the transcription of antioxidant genes [59].

Besides Nrf-2, FOXOs are downstream effectors of SIRT1 stimulation [60,61] and their activity can be modulated through deacetylation, as well as dephosphorylation. FOXOs promote the nuclear transcription of genes able to drive ROS detoxification. Several works suggest that FOXO family, in particular isoform FOXO3, is required for lifespan prolongation in mice undergoing dietary restriction. In this condition, it promotes the transcription of those genes involved in oxidative stress resistance, including the enzymes catalase and the manganese-dependent superoxide dismutase (MnSOD), which represent crucial antioxidant mechanisms for maintaining vascular homeostasis [50,62–64].

As regards AMPK, it orchestrates many biochemical events including glucose uptake, glycolysis, oxidation of free fatty acids and mitochondrial biogenesis [65]. Interestingly, AMPK activation contributes to raise ATP levels and restore myocardial contractile efficiency and vascular response. Moreover, it also activates eNOS and prevents mitochondrial insufficiency, inflammation and cellular death [66]. Noteworthy, AMPK also regulates, by phosphorylation, mTOR, modulates the insulin-like growth factor-1 (IGF-1) pathway and controls SIRT1

activity by regulating the levels of NAD^+ and its regulator nicotinamide phosphoribosyltransferase (Nampt) [67–69]. In animal models, the activation of AMPK reduced pressure overload–induced cardiac hypertrophy and played anti-ischemic cardioprotective effects [70]. Moreover, AMPK activation has shown to diminish SA-βgal staining [71]. Interestingly, being a metabolic sensor, AMPK is stimulated during caloric restriction [72]; thus, pharmacological modulators may represent useful tools to prevent ageing-related features.

mTOR is an evolutionarily conserved serine-threonine kinase that senses and integrates diverse environmental and intracellular signals, such as those initiated by growth factors and nutrients, to direct cellular and organismal responses [73]. Also mTOR is tightly related to caloric restriction [74] and has been recognized as a regulator of lifespan in the nematode *Caenorhabditis elegans* [75], in the fruit fly *Drosophila melanogaster* [76] and in the yeast strain *Saccharomyces cerevisiae* [77]. Interestingly, the inhibition of mTOR by rapamycin doubles the lifespan of these simple organisms [78,79].

Recently, a main role of mTOR in inflammation, through the promotion of SASP, and in the increase in mitochondrial mass has been hypothesized, thus suggesting that dysfunctional mitochondria can cause mtDNA damage [80].

Finally, IGF-1 is a growth factor that regulates downstream phosphorylation cascade including the mTOR pathway; however, its role in ageing is largely unknown and conflicting results have been reported [81].

4 Role of hydrogen sulfide in ageing

Besides the above intracellular pathways, more recently H_2S has emerged as a further key component, whose decline has been associated to ageing. Indeed, a perturbation of H_2S signaling is involved in a wide range of age-related diseases [82]. Although many mechanisms underlying the pleiotropic effects of H_2S are still unclear, persulfidation (or S-sulfhydration) of relevant target proteins is the most plausible explanation for beneficial effects of this gasotransmitter in extending lifespan. Worthy of mention, Zivanovic and colleagues, developing a new method to selectively label S-sulfhydration proteins, elegantly highlighted a decline of protein persulfides in Wistar rats during ageing. Interestingly, they found a persulfidation approximately 50 % lower in 24 month old rats if compared with 1 month old rats. Accordingly, a decrease in persulfide levels was displayed in human fibroblasts obtained from the same individuals but collected at different ages (31 and 48 years of age). The same authors observed, by using of 7 and 20 month old mice fed *ad libitum* or subject to daily caloric restriction (from 2-month-old), that liver persulfidation levels declined with ageing, but higher levels were found in caloric restriction group. Indeed, an intrinsic feature of persulfides is that they may react with ROS and thus may represent a protective mechanism against oxidative stress. Finally, the proof of the concept was achieved in *C. elegans*. Indeed, worms growing in a medium supplemented with thiosulfate (an H_2S-mimetic agent) had a significant increase (about 15 %) in median longevity [83]. Other studies confirmed that H_2S is responsible for increased longevity afforded by caloric restriction in yeast, *C. elegans*, *Drosophila melanogaster* and mice [84,85]. Qabazard et al. observed that the H_2S-donor GYY4137 (Fig. 3) increased the expression of several antioxidant genes in *C. elegans* and additionally increased their lifespan [86]. Therefore, these recent findings support the hypothesis that H_2S modulation is a new target to maintain redox homeostasis and can represent a promising strategy to enhance health-span.

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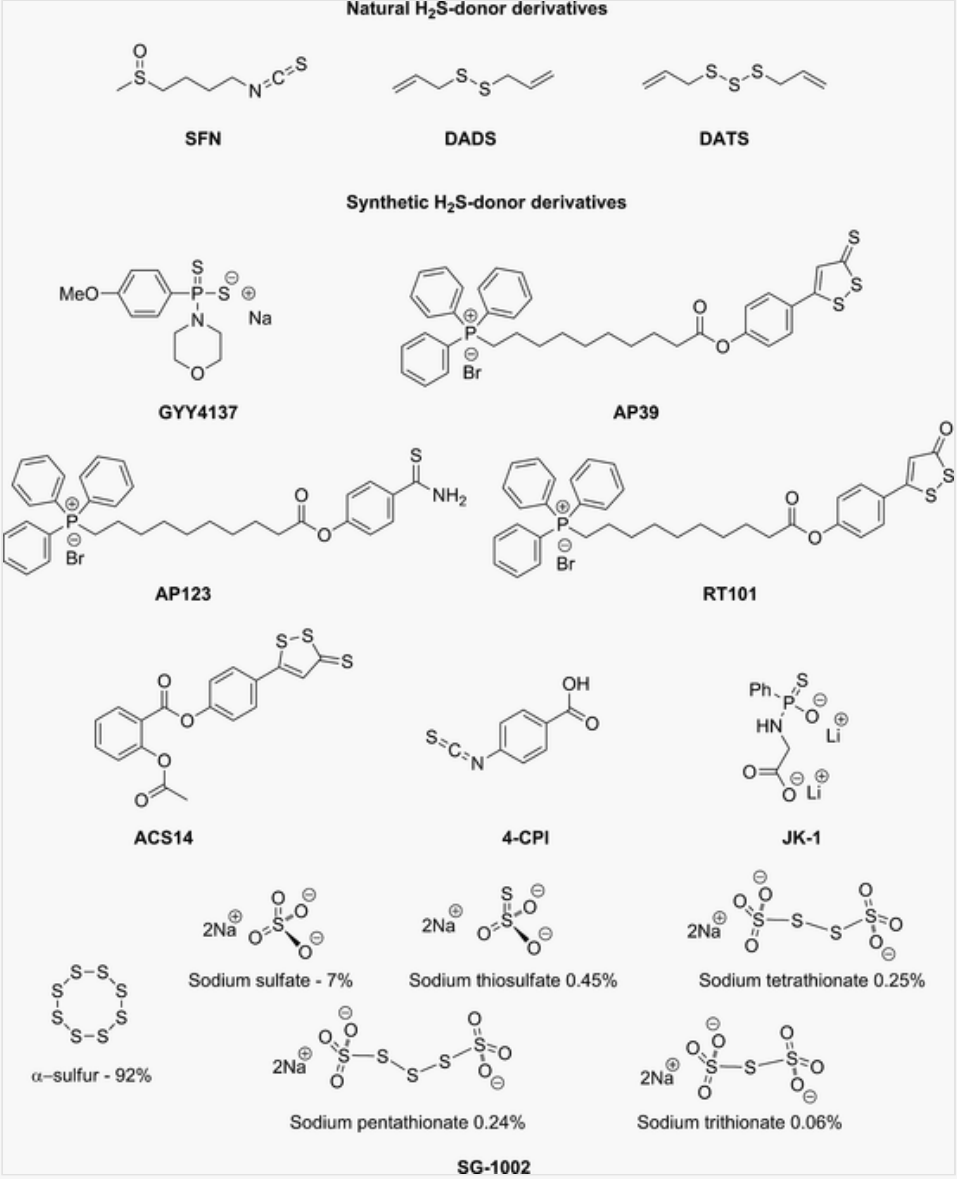
Fig. 3



Chemical structures of H_2S-donor compounds.
Chemical structures of H_2S-releasing drugs used for characterizing the role of H_2S in cardiovascular ageing are depicted. In particular, natural-derived, diallyl disulfide (DADS), diallyl trisulfide (DATS) and sulforaphane (SFN) and synthetically-derived H_2S donors, GYY4137, AP39, AP123, RT-101, 4CPI, SG-1002 and ACS14, are reported.

4.1 Biosynthesis and catabolism of H_2S

In mammalian tissue, H_2S is biosynthesized through enzymatic and non-enzymatic ways. The non-enzymatic pathway represents a less important way and consists in the reduction of elemental sulfur to H_2S with the oxidation of six molecules of glucose. As concerns the enzymatic way, it starts from a common substrate, the aminoacid ~~L~~L-cysteine and is mainly managed by two cytosolic, pyridoxal-5′-phosphate-dependent enzymes: cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS). Even if these two enzymes are largely expressed in several tissues, CBS is predominant in central nervous system (CNS) while CSE is mainly involved in H_2S biosynthesis at cardiovascular level [87]. Besides these two mainly-involved enzymes, other two enzymes cooperate to the H_2S production such as 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT), which originally were identified only as mitochondrial enzymes but recently were discovered also in the cytosol [88]. In particular, as concerns cardiovascular system, CSE mRNA was first discovered in VSM but currently it has been demonstrated that CSE is predominantly expressed in vascular endothelium where H_2S is biosynthesized and then it spread as an endothelial-derived relaxing factor towards VSM. As a further confirmation of the predominance of CSE at endothelial level, in CSE knockout mice

endothelial dysfunction, with consequent hypertension and atherosclerosis, has been observed [89–92]. On the other hand, as already observed for H_2S biosynthesis, also H_2S degradation can follow different ways: first of all, H_2S is a reducing agent and so it is frequently consumed by several oxidant factors continuously present in many tissues, but probably the main catabolic way for H_2S is represented by its oxidation at mitochondrial level, where H_2S is rapidly converted to sulfate and sulfite species through the sequential action of quinone oxidoreductase, rhodanese and sulfur dioxygenase. Other less important catabolic processes for H_2S are represented by the reaction between H_2S and methemoglobin which results in sulphemoglobin formation and the reaction between H_2S and thiol S-methyltransferase (TSMT) which gives methanethiol and then dimethylsulphide [93].

5 Hydrogen sulfide and molecular targets involved in cardiovascular ageing process

5.1 Hydrogen sulfide anti-ageing effects through the modulation of SIRT1

Intriguing overlapping between SIRT1 activation obtained through a caloric restriction and the effects observed after H_2S exposure has been highlighted in many studies. Interestingly, H_2S is able to directly activate SIRT1 enzyme, suggesting that it may be involved in extending life span and delaying the cardiovascular ageing [94]. Miller and Roth in 2007 reported that nematodes exposed to H_2S became healthier and showed a longer lifespan if compared with untreated worms [95].

Furthermore, caloric restriction positively modulates H_2S-generating enzymes (CSE and CBS) in mice, leading to increased H_2S endogenous production and significant decrease of oxidative stress. Thus, caloric restriction may be helpful to maintain H_2S physiological levels during the ageing process, and assure a quite activation of SIRT1 [96]. Recently, it has been demonstrated that H_2S counteracts cell senescence in an *in vitro* model of ageing, by using human umbilical vein endothelial cells (HUVEC), suggesting the involvement of SIRT1 pathway [97]. Taken together, these evidence lead to conclude that H_2S can be considered as a caloric restriction-mimetic agent, endowed with clear anti-ageing properties mediated by the activation of SIRT1 (Fig. 4). In addition, recently Du and colleagues demonstrated that the anti-atherogenic effect of NaHS or GYY4137 was mediated by SIRT1 stimulation. Indeed, both the treatment with H_2S-donors and the overexpression of CSE was associated to an increase of SIRT1 expression. Interestingly, the authors also demonstrated that this event was linked to S-sulphydration of two CXXC domains, which are responsible for the binding of SIRT1 to zinc, and then contribute to its structural stabilization [98].

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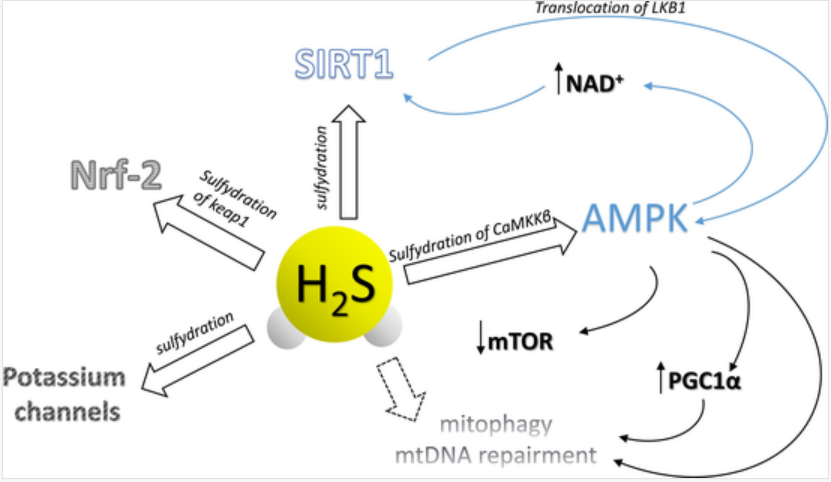
Fig. 4



Intracellular ageing-related pathways influenced by H_2S.
H_2S, through sulfydration of cysteine residues of target proteins, can modulate the main actors of ageing. In particular, H_2S can directly and indirectly stimulate SIRT1. Nrf-2 also is activated by sulfydration of keap-1, and thus favoring its nuclear translocation. AMPK can be indirectly stimulated *via* CAMKKβ and *via* translocation of LBK1. The stimulation of AMPK contributes to modulation of mitophagy and mtDNA reparation. Finally, H_2S can direct operate a sulfydration of potassium channels.

The binomial "H_2S and SIRT1" has been also suggested in an *in vitro* model of cardiomyocytes submitted to oxidative damage induced by H_2O_2. Indeed, NaHS exposure limited ROS production in H9c2 cardiomyocytes and increased the expression and activity of SIRT1, related with higher levels of SOD and glutathione S-transferase (GST) [99]. The co-administration of NaHS and Ex 527 (SIRT1 inhibitor) almost completely abolished the beneficial effect mediated by NaHS, confirming that the main molecular target mediating H_2S antioxidant effect is the SIRT1 pathway [100]. Likewise, NaHS exposure increased SIRT1 activity in senescent endothelial cells treated with H_2O_2, although the expression of SIRT1 was not altered [101]. Suo and colleagues proposed a direct stimulation of SIRT1 by H_2S; however, it may indirectly activate the enzyme even by increasing NAD^+ levels [102]. Consistently, diallyl trisulfide (DATS) (Fig. 3), an organosulfur compound of garlic, widely described in the scientific literature as a natural H_2S donor [103], promoted the up-regulation of cardiac SIRT1 expression in a mouse model of I/R injury, limiting the production of ROS during the reperfusion phase and inhibiting endoplasmic reticulum (ER) stress-dependent apoptosis [104].

5.2 Hydrogen sulfide anti-ageing effect through the modulation of Nrf-2

A body of evidence suggests that H_2S plays a pivotal role in the antioxidant machinery, in fact an impairment in physiological H_2S production causes severe imbalance between antioxidant molecules and ROS generation, leading to high levels of oxidative stress associated with early cell senescence [105]. Such a condition represents a risk factor for the development of CVDs, for example atherosclerosis and hypertension, since early vascular cell senescence may compromise endothelium integrity. In this context, H_2S-donor treatment shows beneficial

effects in those pathological conditions related to high ROS levels and oxidative damage. Interestingly, Nrf-2 is recognized as one of the molecular targets of the H_2S-mediated antioxidant response. Indeed, H_2S directly interacts and modulates Nrf-2 by its post-translational modification due to S-sulfhydration. Despite various studies early suggested a direct interaction of H_2S with Nrf-2; more recently the exact mechanism of action has been demonstrated [106]. In particular, Yang and colleagues observed that H_2S S-sulfhydrates the cysteine-151 residue of Keap1, triggering the Nrf-2 dissociation, its nuclear translocation, and finally the expression of antioxidant genes (Fig. 4). Mutations on cysteine-151 residue with alanine completely abrogated the Keap1 sulfhydration and the antioxidant defenses [107]. Likewise, sulforaphane (SFN), a thiol-containing compound of *Brassicaceae* family (Fig. 3), is recognized as a potent antioxidant. It is able to modify Keap1 at cysteine-151 residue level. Indeed, in an oxidative stress-induced senescent model, SFN promoted the activation of Nrf-2, but not its expression, and ameliorated the antioxidant machinery [108,109]. Noteworthy, SFN is an isothiocyanate recently recognized as a smart H_2S-donor [110–112]; therefore, the beneficial anti-ageing effects observed with this molecule are very likely to be ascribed to H_2S. SFN treatment (0.5 mg/kg for five days in each week for 3 months) showed increasing expression of Nrf-2 and its nuclear localization in diabetic mice [113], resulting in a substantial prevention of diabetes-induced development and progression of cardiac dysfunction and remodeling. The cardiac protection was even more significant at the 6th month in treated diabetic mice. These data strongly suggested that SFN prevents cardiomyopathy by up-regulating of Nrf-2 expression and function [114]. Furthermore, SFN was found to dose-dependently (0.1–5 μmol/L) enhance the viability of cardiomyocytes and decrease apoptotic cells by suppressing caspase-3 activity. This effect leads to a marked reduction of the expression of ER stress-related apoptosis proteins by SFN, elevating the expression of SIRT1 and Bcl-2/Bax ratio [115].

5.3 Hydrogen sulfide anti-ageing effect through the modulation of AMPK

In 2013, Zhang and colleagues demonstrated that H_2S inhibited AMPK, impairing the glucose utilization at hepatocytes level [116]. However, more recently this hypothesis was confuted and the idea that H_2S activates AMPK is presently accepted [117]. As reported by Wang et al., AMPK can be considered as a main mediator of H_2S-associated cardiovascular beneficial effects. In particular, *via* AMPK, H_2S showed cardioprotection against I/R injury, as well as cardioprotective effects in high fat diet-induced cardiac dysfunction [118–120]; as well, protection against diabetic complications and overall anti-inflammatory and anti-oxidant effects were attributed to the stimulation of AMPK [120]. Likewise, the H_2S-donor GYY4137 protects H9c2 cells against hyperglycemia-induced cytotoxicity by activation of the AMPK/mTOR signal pathway [121].

Accordingly, H_2S limits endothelial dysfunction in diabetic conditions, which leads to the impairment of vasodilation response and the increase of ROS production. Indeed, the exogenous administration of H_2S-donors preserved endothelial cells against hyperglycemia damage by activating AMPK signaling pathway, that consequently lead to mTOR inhibition activity [122,123]. The beneficial effects of well-known AMPK-activators (i.e. metformin) are widely reported; noteworthy, H_2S mimics those beneficial effects typical of AMPK activation. Hyperglycemia is considered a condition predisposing to early ageing associated with endothelial dysfunction and vascular inflammation; both these conditions lead to ageing process due to high formation of ROS. H_2S-donors or L-cysteine, endogenous precursor of H_2S, significantly reduced high glucose-mediated ROS increase and secretion of inflammatory cytokines and such beneficial effects were mediated by the activation of AMPK [117,124]. Hypertriglyceridemia, which probably represents one of the most diffuse metabolic diseases, is a further pathological condition characterized by early age-related CVDs. In such a pathological condition, lower endogenous levels of H_2S were found both in high fat-fed animals and hypertriglyceridemic patients. Conversely, the administration of NaHS reduced triglyceride plasma levels and

counteracted hepatic steatosis. Interestingly, these beneficial effects are due to induction of autophagy through the activation of AMPK and the consequent inhibition of mTOR [125]. As regards the specific mechanism of H_2S-induced AMPK activation, it has been proposed that the gasotransmitter may act via calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) pathway. Indeed, in *in vivo* model of ageing induced by treatment with galactose, an up-regulation of phosphorylated AMPK, as well as other kinases related to ageing (for instance, Akt and PI3K) was found. Conversely, the phosphorylation of AMPK drastically decreased when animals were treated with an inhibitor of CAMKKβ, demonstrating that this is the way through which H_2S plays preservation of mitochondrial function and reparation of mtDNA damage [126,127] (Fig. 4). Besides there is a novel intriguing link between SIRT1/AMPK in the regulation of the ageing process represented by liver kinase B1 (LKB1), a target of SIRT1. Cellular activity and localization of LKB1 depends on the degree of acetylation and deacetylation. In particular, SIRT1-mediated deacetylation favors the translocation of LKB1 from the nucleus to the cytoplasm and therefore its activation, following the formation of a complex with STRAD and/or MO25 that, in turn, it is able to phosphorylate AMPK [127]. Interestingly, Silvestre and colleagues defined also the presence of mutual regulation between SIRT1 and AMPK. AMPK increases the activity of SIRT1 by activating Nampt, while SIRT1 favors the action of AMPK through the deacetylation of LKB1 (Fig. 4). In general, Nampt catalyzes the formation of NAD^+ starting from NAM and, thus increases the activity of SIRT1, which is a NAD^+-dependent enzyme [128]. Consistently, the administration of the H_2S-donor SG-1002 (Fig. 3) influenced the nuclear translocation and transcriptional activity of PGC-1α, regulated by post-transcriptional modifications (e.g. phosphorylation mediated by AMPK and deacetylation through the cooperation of SIRT1) [129]. In AMPK-knockout mice the addition of SG-1002 did not alter the expression of PGC-1α target genes and in mitochondrial DNA levels, thus underlining that the effect of H_2S as an inducer of mitochondrial biogenesis occurs through the AMPK-SIRT1/PGC-1α axis [130].

5.4 Hydrogen sulfide anti-ageing effect through the modulation of potassium channels

Consistent with the role of channelopathies in ageing, H_2S has been demonstrated to finely modulate potassium channels. H_2S has been firstly characterized as an opener of K_{ATP} (ATP-sensitive potassium channels). This channel plays an important role in the cardiovascular activity of H_2S, since it promotes vasodilation and triggers cardioprotective effects against I/R injury [131,132]. Later, other potassium channels have been recognized as a target of H_2S, including voltage-gated potassium 7.4-channels (Kv7.4), responsible for vasorelaxing effects [133]. Presently, the role of H_2S–mediated K_{ATP} channel activation in ageing is poorly understood. However, a recent study about the involvement of H_2S in the modulation of K_{ATP} channels in aged porcine oocytes paves the way to possible intriguing perspective. In fact, Nevorel and colleagues demonstrated that H_2S exposure protects against undesired phenotypic changes correlated with ageing process in oocytes, by activation of the K_{ATP} channels, probably through the S-sulfhydration of cysteine thiols [134] (Fig. 4).

5.5 Hydrogen sulfide anti-ageing effect through the modulation of mitophagy

As discussed, ageing represents a progressive decline that interests many organs and their physiological functions. This decline is associated with an increased possibility to develop different kind of disorders, including cardiovascular age-related diseases. The senescence causes in the heart a LV fibrosis and diastolic dysfunction with an increase to heart failure [25]. Among the mechanisms implicated in cardiac ageing, it has been observed that mitochondrial oxidative stress and mitochondrial dysfunction play a pivotal role in senescence-related CVDs [135]. These functional mitochondria impairments can be resolved by a mechanism namely mitophagy (selective degradation of mitochondria by autophagy). This highlighted a strength interplay between autophagic machinery, ageing and consequently age-related CVDs [136], indicating that autophagy is

of vital importance in maintaining healthy cardiovascular functions. Numerous studies showed that H_2S can exert a significant protective role in the heart even by modulating autophagy [137], although the results are somewhat conflicting. Briefly, in *in vivo* model of cardiomyopathy, H_2S ameliorated myocardial fibrosis by downregulating myocardial autophagy [138]. On the other hand, it has been observed that, H_2S protects myocardiocytes in an animal model of type 2 diabetes model by promoting autophagy [121]. SIRT1/mTOR signaling could represent one of the pathways involved in H_2S-mediated autophagy promotion and cardiovascular ageing retardation (Fig. 4).

On the basis of the role of H_2S in protecting cardiovascular system, it has been hypothesized that this gasotrasmitter could be a key component for maintaining healthy functions in ageing cardiovascular tissue. Accordingly, Talaei and co-workers [139] observed that genes levels expression of CBS, CSE, and SIRT-1, involved in maintaining the proliferative capability by overcoming senescence, were highly reduced in a variety of prematurely senescent cells in which mTOR expression was promoted. The administration of NaHS (50 μM) was able to revert this condition precluding the activation of mTOR and increasing SIRT-1 expression and the LC3-II/LC3-I ratio, which modulates the autophagy levels, preventing premature cellular ageing. Thus, H_2S increases the expression of SIRT-1, repressing mTOR-activated autophagy, and ultimately protected against cardiovascular ageing [140]. In another work, Chen and colleagues [120] demonstrated that exogenous H_2S-donors caused the rescue of cardioprotection from ischemic post-conditioning (IPostC) by increasing autophagy in aged hearts. For this study, they used aged rat hearts (24-months-old) and cardiomyocytes (where senescence was induced by ~~D~~D-galactose) exposed to an I/R and IPostC protocol. They observed that IPostC protection was missing in cardiomyocytes and aged heart, but could be restored by NaHS (10 μM). This latter by upregulating the AMPK/mTOR pathway is able to reduce myocardial injury, infarct size and apoptosis improving cardiac functions, by increasing cardiac cell viability and by modulating autophagy in the aged heart. Indeed, the administration of the autophagy inhibitor 3-methyladenine, inhibited the beneficial role of H_2S on IPostC-induced cardioprotection in aged cardiac cells. Accordingly, H_2S-mediated upregulation of autophagy in the aged heart and cardiomyocytes, could restore the protective effect of IPostC on the myocardium via AMPK/mTOR pathway. Interestingly, in this work, H_2S was described as a modulator able to activate autophagy in myocardial I/R events and to inhibit it during processes of cardioplegia and cardiopulmonary bypass. Latorre and colleagues in an interesting work [141], following the observations that H_2S could exert a protective role in many tissues (including cardiac tissue) against senescence by targeting mitochondria, reported that exogenous H_2S attenuates endothelial senescence by selectively promoting splicing factors *HNRNPD* and *SRSF2*. In particular, it has been demonstrated that, during ageing, different biochemical and functional pathways undergo an aberrant regulation in the human peripheral blood transcriptome. These pathways are enhanced for transcripts encoding the regulatory apparatus that governs splice site choice. Notably, changes in the regulation of splicing seem to be crucial in lifespan of mammals [142], since it was demonstrated that many age-related disorders (i.e. Alzheimer's and Parkinson's diseases) are characterized by an aberrant regulation of alternative spicing. So, maintaining a healthy splicing regulation throughout the life course could prevent cellular senescence and age-related diseases [143]. Remarkably, also cardiomyocyte senescence leads to vascular dysfunction and increased vascular risk including heart failure [40]. Interestingly, senescent cardiomyocytes showed dysregulation of splicing regulator expression [144]. In the mentioned study the researchers evaluated the effect of different exogenous H_2S-donors on splicing regulatory factor expression and cell senescence phenotypes, in senescent primary human endothelial cells. They employed a widely used H_2S-donor, GYY4137. Furthermore, they employed other three H_2S-donors (AP39, AP123 and RT01, Fig. 3) previously demonstrated to specifically target the mitochondria [145,146]. They used 100 μg/mL of GYY4137 and 10 ng/mL for the mitochondrially-targeted H_2S donors AP39, AP123 or RT01. To assess the influence of H_2S-

donors on splicing regulators, the splicing factor expression was measured by qRT-PCR in senescent cultures of human aortic endothelial cells (HAoEC) following 24 h~~ours~~ treatment with AP39, AP123, RT01 and GYY4137. The treatment with GYY4137 caused a general upregulation of the expression of splicing factor in treated cells. The other mitochondrion-targeted H_2S-donors were able to rescue HAoEC from senescence. Interestingly, this kind of compounds specifically upregulated transcripts encoding the splicing activator SRSF2 and the splicing inhibitor HNRNPD. Abolition of either *SRSF2* or *HNRNPD* expression in primary endothelial cell lines, in the absence of any treatment, led to an increase of cellular senescence. These results indicated that mitochondria-targeted H_2S could be a promising tool for reverting senescence phenotypes.

6 Contribution of H_2S in the ~~Prevention of Ageing-Related Cardiovascular D~~prevention of ageing-related cardiovascular diseases

6.1 H_2S and vascular function

The vascular function of H_2S was first associated with its ability to induce vasodilation through the opening of the K_{ATP} channels [133,147], the activation of voltage-gated potassium channels (Kv7) [133,148] and the inhibition of 5-phosphodiesterase (5-PDE) [149] and consistently many synthetic and botanical H_2S-donors have been described as vasodilators [103,104,150–155]. However, the action of H_2S at the vascular level is more complex and relies also on its ability to counteract inflammatory and oxidative stimuli. As concerns the anti-oxidant activity, it is widely recognized that H_2S acts as an anti-oxidant agent, not only because of the chemical feature of reducing agent, but also because it is able to activate several antioxidant machineries, such as the Nrf-2/Keap-1 pathway or one of the most important key factor of the antioxidant/anti-ageing pathway, that is SIRT1 [102]. Recent studies focused their attention on the role of H_2S at the endothelial level and showed that the administration of NaHS induced the increase of intracellular NAD^+ amount, the improvement of endothelial cells motility and migration, and the induction of the spheroid-sprouting phenomenon, in a SIRT1 dependent manner with a consequent increase of vascular endothelial growth factor (VEGF) levels [94,156]. Moreover, investigating the effect of the nutrients restriction on the health of the vascular tree, Hine and colleagues discovered that H_2S inhibited, in a transient manner, the mitochondrial respiration, acting on the electron transport chain; this inhibition activated another key factor in cellular metabolism, i.e. AMPK which promotes glucose uptake and utilization in endothelial cells, leading to an increased glycolysis [157]. Taken together these findings suggest that H_2S, through the increase of VEGF levels and the activation of AMPK, leads to endothelial cells proliferation, migration and angiogenesis, which are fundamental processes to prevent or reduce the vascular ageing [158]. Furthermore, the protection of the endothelial tissue was demonstrated also in ~~D~~D-galactose-challenged HUVECs and in ~~D~~D-galactose-induced senescent mice. Indeed, in these two experimental models, an increase of endogenous anti-oxidant factors and a reversion of the ~~D~~D-galactose accelerated ageing, were observed after administration of exogenous H_2S [159]. An interesting study by Berenyova and co-workers on normotensive and spontaneously hypertensive rats (SHR), highlighted that there is a cross-talk between H_2S and NO and in particular they found that in young normotensive animals with intact vessels, H_2S induced a paradoxical pro-contractile effect, probably due to its scavenging action on the NO production. On the other hand, in vessels affected by endothelial dysfunction, from old SHRs, in which there is a deficiency of the NO pathway, the administration of H_2S induced a vasorelaxant response confirming a role of vicarious for H_2S in the maintenance of vascular function when NO decreases, as a consequence of CVDs and ageing [160]. A similar observation, on the emerging importance of H_2S in dysfunctional vascular beds, has been reported by Testai and colleagues on the coronary system of SHRs, which are a recognized model of endothelial dysfunction

and consequent NO deficiency [161]. However, although a crosstalk between H_2S and NO has been established, unclear mechanisms - beyond the objectives of this review - contribute to these effects, making this scenario nebulous.

As a further beneficial effect on the vascular tree, H_2S exhibited a clear inhibition of the vascular inflammation phenomenon. Indeed, in an elegant work by Bibli and co-workers, the authors demonstrated that the exogenous administration of a polysulfide H_2S-donor reverted the endothelial inflammation and the atherosclerotic process associated to the deletion of endothelial CSE [162]. Moreover, in endothelial cells, H_2S showed the ability to reduce the tumor necrosis factor alpha (TNF-α)-induced increase of inflammatory mediators, such as vascular cell adhesion protein 1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1), confirming its protective role on vessels wall against vascular inflammation [163,164].

6.2 Hydrogen sulfide and atherosclerosis

Notably, H_2S exhibits several properties which may be useful to prevent and to manage the onset of atherosclerosis and the formation of the atherosclerotic plaque. Indeed, low amounts of H_2S counteract ROS and oxidant events in VSM. In particular, H_2S inhibits one of the most important step in the onset of atherosclerotic modifications of the vascular wall, that is the oxidation of LDL operated by oxidant agents like hypochlorite [165]. Subsequent studies demonstrated that H_2S has a role in the atherosclerotic process, also through the inhibition of another fundamental step: the expression of the ICAM-1 both in HUVEC and in apoE$^{-/-}$ mice [166]. But H_2S is also able to reduce the processes of intima and VSM cell proliferation, which is a typical event of atherosclerotic plaque formation and development [167,168]. Finally, H_2S seems to be fundamental also in late phase of the atherosclerotic process: angiosteosis and calcification. Indeed, a down-regulation of the CSE expression and an impairment of the CSE activity were found in calcified vessels and, at the same time, the administration of the H_2S-donor NaHS reduced the calcium accumulation and the expression of genes encoding for osteopontin, a glycoprotein involved in biomineralization processes [169]. On the basis of these first findings, the use of molecules able to release H_2S seems to be an interesting strategy in order to prevent and also to treat both the early and the advanced phases of atherosclerosis. Moreover, the synthetic H_2S-donor GYY4137 showed the ability to inhibit the oxidized LDL-induced foam cell generation in human macrophages and the expression of pro-inflammatory factors like ICAM-1, NF-κB and chemokine both in macrophages and in *in vivo* apoE$^{-/-}$ mice fed with high fat diet [170]. Similar results have been obtained using a particular type of "H_2S-hybrids". These hybrids are original molecules composed by a native well-known drug conjugated with a H_2S-donor moiety [171–173], in order to improve the native drug pharmacodynamic profile, following an intriguing strategy, which has been previously used for the most known gasotransmitter NO [91,174–177]. In particular, as concerns the hybrid H_2S-donors tested in atherosclerosis process, Zhang and co-workers found that the compound named ACS14 (Fig. 3), i.e. H_2S-releasing aspirin, downregulated the CX3C receptor 1 both in stimulated macrophages and in arteries of apoE$^{-/-}$ mice fed with high fat diet, and this CX3CR1 downregulation correlate with the prevention of atherosclerosis [178]. Among the several available H_2S-donors, botanicals represent one of most promising class and in particular, the garlic polysulfides, like diallyl disulfide (DADS, Fig. 3) or DATS (Fig. 3), exhibited protective effects against the deleterious consequences of LDL oxidation, such as the inhibition of VCAM-1 and E-selectine expression and the consequent monocyte adhesion on endothelial cells [179,180]. Finally, isothiocyanates, both synthetic and deriving from the enzymatic hydrolysis of glucosynolates contained in *Brassicaceae* (or Crucifers) family, represent another group of H_2S-donors [110,111,152,181]. Among them, SFN is currently the most investigated in several diseases. As concerns atherosclerosis, it is able to inhibit the vascular inflammation and the expression of adhesion molecules in HUVEC cells and in rat aortas stimulated with advanced glycation end products (AGEs). Moreover, SFN, when

administered *in vivo* to high fat diet-fed rabbit, reduced the intima/media ratio and decreased the levels of several deleterious factors like LDL, C-reactive protein or NF-κB and, at the same time, increased high density lipoprotein (HDL) levels and improve the endothelium-dependent vasorelaxation in aortas treated with acetylcholine, confirming the protective role of isothiocyanates against the atherosclerotic damage [182].

6.3 Hydrogen sulfide and myocardial ischemia

Many experimental and epidemiological data correlate an increase of deaths due to myocardial injury with age, suggesting that ageing compromises the cardiac resistance against ischemic insults and reduces the endogenous cardioprotective defenses [183]. Furthermore, myocardial infarction is well established to be an event stimulating the tissue destruction and often represents the way to heart failure [184]. In this context, Testai and colleagues observed that the administration of H_2S, by using the H_2S-donor 4-carboxy phenyl-isothiocyanate (4CPI, Fig. 3), significantly protected the cardiac tissue from injury and allowed a remarkable recovery of the post-ischemic myocardial functionality in isolated rat heart subjected to I/R process. In this study, the authors identified the opening of mitoK$_{ATP}$ channels and the reduction in ROS as the main mechanisms of action [185,186]. Other preclinical evidence emerged about the role of H_2S in IPostC myocardial protection, indeed generally IPostC induces protective effects in isolated young rat hearts but not in aged hearts. However, Chen and colleagues demonstrated that the administration of exogenous H_2S was able to recover the protective effect of IPostC against I/R events also in aged cardiac cells (H9c2) and in isolated aged (24-month-old) rats hearts [120]. It is likely that DATS administration reduced infarct size and improved contractile function after acute myocardial I/R injury by restoring cardiac H_2S levels [187]. Beside strong evidence of short-time cardioprotective effects, more recently evidence of long-time effects was reported. Interestingly, although a single administration of H_2S at the time of reperfusion is beneficial in attenuating infarct size, a daily H_2S therapy initiated at the time of reperfusion and continued for several days after reperfusion provided significant improvements in cardiac function, suggesting a positive effect in cardiac remodeling consequent to myocardial infarct [188].

6.4 Hydrogen sulfide and fibrosis and heart failure

Myocardial fibrosis is a typical event of ageing and a hallmark feature of heart failure. During the progression of myocardial fibrosis, various immunological and molecular mechanisms are engaged. Beyond Angiotensin II, transforming growth factor-β 1 (TGF-β1) is a well-known fibrogenic growth factor involved in cardiac fibrosis, capable to trigger inflammation and stimulate the deposition of collagen [189,190].

Interestingly, H_2S has been found to suppress TGF-β1-induced differentiation from fibroblasts, showing anti-fibrotic effects [191,192]. Noteworthy, the involvement of H_2S in the preservation of heart from cardiac ageing and degenerative processes was first demonstrated by Jin et al., who recorded decreased levels of H_2S in plasma, heart tissue and urine and an alteration of the expression of the H_2S-producing enzymes, in mice treated with a 30 %-fructose solution for 15 months, in order to induce a severe diabetic condition associated with cardiac fibrosis, hypertrophy and dysfunction [193].

Further evidence has been obtained with GYY4137, that decreased myocardial fibrosis, through the block of TGF-β1/Smad2 signaling pathway and decrease in expression of α-smooth muscle actin (α-SMA) in cardiac fibroblasts [194]. Likewise, S-propargyl-cysteine (ZYZ-802, able to increase the expression and activity of CSE protein and thus endogenous H_2S concentrations) and its liposomal formulation (DP-ZYZ-802) caused an inhibition of myocardial fibrosis *via* TGF-β1/Smad signaling pathway [195,196].

Noteworthy, low levels of circulating H_2S have been found in heart failure patients, leading to hypothesized that this gasotransmitter is critical in the cardiac homeostasis. Li and colleagues demonstrated that a delayed treatment with a recently characterized H_2S-donor, JK-1 (Fig. 3), reduced the over-activation of the sympathetic nervous system and renin-angiotensin-aldosterone system, possibly through enhancing NO bioavailability and signaling, and reducing oxidative stress in a well-established pressure overload HF model [197]. Furthermore, SG-1002 prevented cardiac dilatation, preserved LV function and reduced hypertrophy in an aortic constriction-induced model of heart failure [198]. Currently, SG-1002 is in a phase II clinical randomized trial, on 50 patients for treatment of heart failure. Interestingly, previous studies confirmed a good tolerance and a significant efficacy; in particular SG-1002 increased circulating levels of H_2S and importantly stabilized serum brain natriuretic peptide (BNP, a marker of the severity of heart failure) levels [199].

In this scenario, an interesting contribute of angiogenesis has been also supposed. Indeed Calvert's group observed that H_2S therapy with DATS improved LV remodeling and preserved LV function in a model of transverse aortic constriction, at least in part, through increase the expression of the proangiogenic factors, including vascular endothelial cell growth factor and the bioavailability of NO [200].

7 Conclusions

In the last decades, the life expectancy in the population of the Western society has drastically increased. Current projections indicate that such a life prolongation will follow a constant trend again for the next decades. The demographic consequences of this phenomenon have (and will increasingly have) epochal consequences. In fact, the percentage of "elderly" individuals in the general population has reached levels never recorded in the past, with important political, sociological and epidemiological consequences [201]. In particular, this has led to a significant increase in the incidence and prevalence of diseases typically associated with ageing. These age-related pathologies are often linked to a progressive dysregulation of the immune system and to a reduced ability to manage the redox balance. Among the various organs and systems affected by age-related pathologies, the cardiovascular system certainly plays a leading role and is the site of important widespread diseases, which dramatically represent today the main cause of mortality [202]. Therefore, the search for pharmacological and non-pharmacological strategies that can promote a "healthy ageing" is currently a timely and compelling issue for the biomedical disciplines. The relatively recent discovery of the "third" gasotrasmitter H_2S, the understanding of its intimate links with multiple molecular mechanisms involved in ageing and inflamm-aging, and the evidence of the plausible relationships between a progressive decline in endogenous H_2S levels and the onset of various cardiovascular age-related diseases paves the way for intriguing prospects. In particular, the heterogeneous armamentarium of H_2S-releasing compounds endowed with different chemotypes and pharmacological features seems to give an original and promising option for preventing, slowing down or treating widespread CVDs associated with ageing.

Declaration of Competing Interest

The authors report no declarations of interest.

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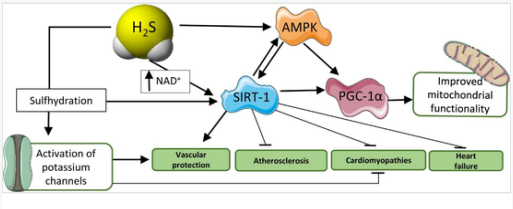

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