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Prospects of Pharmacological Interventions to Organismal Aging

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Abstract: Intense research in the areas of cellular and organismal aging using diverse laboratory model systems has enriched our knowledge in the processes and the signalling pathways involved in normal and pathological conditions. The field finds itself in a position to take decisive steps towards clinical applications and interventions not only for targeted age-related diseases such as cardiovascular conditions and neurodegeneration but also for the modulation of health span and lifespan of a whole organism. Beyond nutritional interventions such as dietary restriction without malnutrition and various regimes of intermittent fasting, accumulating evidence provides promise for pharmacological interventions. The latter, mimic caloric or dietary restriction, tune cellular and organismal stress responses, affect the metabolism of microbiome with subsequent effects on the host or modulate repair pathways, among others. In this mini review, we summarise some of the evidence on drugs that can alter organismal lifespan and the prospects they might offer for promoting healthspan and delaying age-related diseases.

Keywords: TOR; rapamycin; rapalogs; Torin; metformin; insulin growth signalling pathway; aspirin; lifespan.

Introduction

Human life expectancies are steadily rising worldwide, with the demographics of developed countries being shifted towards older ages [1]. This change is accompanied by a disturbing price tag: an increase in occurrence of age-related diseases together with personal, social and financial burdens [2] with 40% of the National Health System's budget spent on over 65s in the UK. Aging research and biogerontology has long been concentrated on the molecular workings underlying the aging phenomenon towards increasing the healthspan and extending the productive timeframes of an individual. Based on various theories of aging [3-6], scientists have focused efforts on processes, genetic, epigenetic or environmental factors that could be setting the pace of the biological clock; or alternatively those that are potentially direct targets, mediators or effectors of this clock. For example, the understanding of the role of nutrition and evolutionarily conserved nutrient-responsive pathways, such as the Insulin Growth Factor (IGF) or the Target of rapamycin (TOR) [7], has been pivotal in linking metabolism, bioenergetics and lifespan. Dissection of these pathways has led us to comprehend genetic perturbations that prolong lifespan, and has provided a picture of the molecular players and functional networks towards developing pharmacological interventions for modulation of stress responses and amelioration or even prevention of age-related diseases. In this mini review we will briefly discuss some of these drugs that clearly extend lifespan or reduce aging rates in diverse model systems. We will refer to signalling pathways such as TOR, AMP-activated protein kinase (AMPK) and IGF. We will also discuss compounds such as metformin or aspirin that are widely used in pathologies as well as dietary supplements such as resveratrol. The effects of these drugs on aging and lifespan are without a doubt impressive based on laboratory model systems data. Although some of them are prescribed for treating diseases (such as metformin for diabetes) their ability to modulate accordingly human aging rates, lifespan and more importantly, healthspan still remains to be demonstrated.

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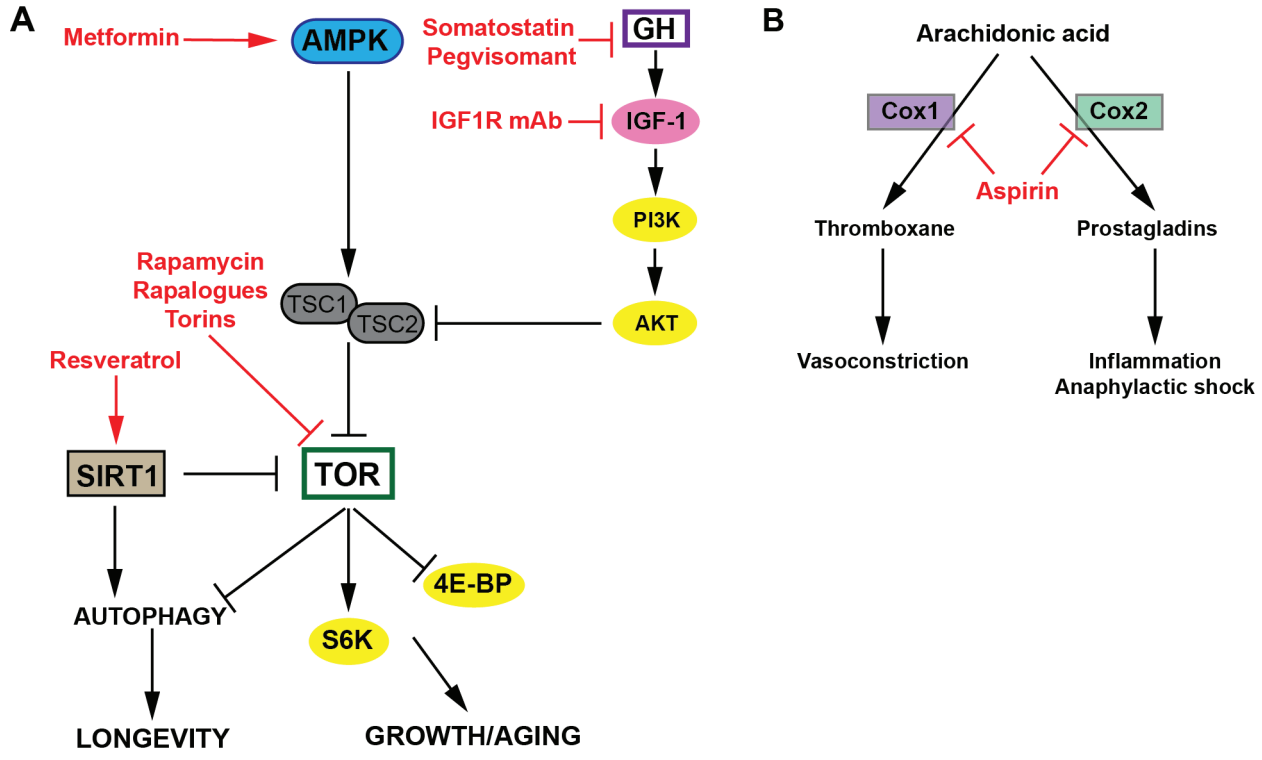

Figure 1: Overview of the signaling pathways controlling cellular growth, metabolism and affecting aging, and some of the drugs mentioned in the manuscript. A. Relationships between IGF/GH axis, TOR and AMPK pathways and sirtuins in aging and lifespan regulation and drugs that either inhibit or boost their functions (look inside text for details). B. General scheme for the inhibitory action of aspirin on COX1 and COX2 leading to beneficial cellular and organismal effects.

Rapamycin, rapalogs and ATP-analogues: inhibiting the TOR signaling pathway

First isolated from the bacteria *Streptomyces hygroscopicus* by Suren Sehgal in 1972, and identified as an antifungal [8], the macrolide rapamycin has been the subject of research interest for nearly 50 years. Initially, rapamycin showed immunosuppressive potential and gained FDA approval for this purpose [9]. It has since been found to be the first pharmacological agent to extend lifespan in both genders of a mammalian species [10]. As its name suggests, rapamycin works by inhibition of the Target of Rapamycin (TOR) pathway which is an evolutionarily conserved nutrient-responsive pathway serving as a cellular rheostat of energy and a regulator of metabolism and growth [11].

Initial discovery of the TOR pathway came in the early 1990s (much later than the discovery of rapamycin) by several methods. Firstly, a genetic screen for *Saccharomyces cerevisiae* mutants identified the gene encoding the cellular receptor for rapamycin, FKBP (FPR1) [12] after the same team had previously identified FKBP as the binding protein for rapamycin's structural homologue FK-506 [13]. While the genes were identified in Heitman's screen, TOR1 and TOR2, the two TOR kinase homologues more commonly referred to as the targets of Rapamycin, were not fully characterised until 1993 and 1994 when TOR2 was identified as a target of rapamycin [14] and TOR1/2 were found to be structurally and functionally similar but non-identical [15].

The importance of these characterisations can only be fully appreciated when viewed within the context of the conservation of TOR from yeast to man. In humans, there is one TOR kinase, known as mechanistic TOR or mTOR [11], rather than the two homologues found in yeast [16]. The isolation of mTOR came in 1994 and marked the first evidence that yeast could be used as a relevant model organism for TOR-related research in humans. mTOR was initially identified as the FKBP-rapamycin-associated-protein (FRAP) [17] but was referred to as mTOR after it was found to be an orthologue to the yeast TOR homologues [18]. Intense research established that mTOR,

as its yeast orthologues, exists and functions within two highly conserved protein complexes termed mTORC1 and mTORC2 [9].

TOR1, TOR2 and mTOR are all considered to be rapamycin targets (Figure 1A) in their respective organisms and, while some key points are known, research into the relationship between TOR inhibition and aging is still ongoing. However, despite the lack of a fully elucidated mechanism, rapamycin and its analogs or 'rapalogs' have long been considered a key candidate for the pharmacological intervention of aging, with strong evidence showing that rapamycin increases the lifespan and healthspan in mouse models [19-24]. Rapalogs hold an advantage over rapamycin itself as a treatment option as they can be developed to have more favourable pharmacological conditions and provide an opportunity for intellectual property, which can be advantageous to the drug development industry [25].

The two complexes, mTORC1 and mTORC2, are differently affected by rapamycin treatment with mTORC1 inhibition occurring immediately and mTORC2 inhibition occurring only after prolonged treatment with the drug [26]. The two mTOR complexes are not only structurally different from one another; they also have distinct differences in their downstream functions. mTORC1 is associated with the control of anabolic and catabolic processes in response to nutrient availability [27] and is much better understood than mTORC2 but it is believed that both could potentially affect healthy lifespan and aging.

Beyond lifespan extension, rapamycin has also shown promise in age-related disease intervention. Age-related diseases pose a threat in much of the developed world where longer lives, poorer diets and lifestyles are considered to be contributing to the rise in these diseases. According to the World Health Organisation there is a distinct gap between life expectancy and healthy life expectancy across the globe. This is an important point to address with pharmacological interventions since the goal would be to extend both the lifespan and healthspan not just increase lifespan at any cost to life quality.

Inflammation is considered to play an important role in many age-related diseases and rapamycin has been shown to have anti-inflammatory effects [27] to the extent of being used as an immunosuppressive drug [9]. Chronic inflammation has been shown to underpin the mechanism of a number of age-related diseases including cancer, dementia and cardiovascular diseases [28]. Dietary and exercise interventions are often advised in these cases however there is a potential for rapamycin to add to these as a pharmacological option.

It is not just inflammation-related mechanisms that rapamycin has shown promise in such diseases. In cancer, rapamycin has been FDA approved for renal cell carcinoma, mantle cell lymphoma, and pancreatic cancer [27]. It is common to see an upregulation of mTOR signalling in cancer patients, creating an interest in mTOR inhibition as a treatment option. However, a major limitation of using rapamycin or rapalogs in this way is that the drugs are often cytostatic rather than cytotoxic causing tumour growth to slow or stop but not reducing tumour size [25].

In Alzheimer's disease and vascular dementia, cerebrovascular dysfunction can be one of the early symptoms used to diagnose the disease [29]. It is also suggested to be a major contributor to disease onset and progression [30]. This highlights the importance of recent findings that mTOR could be a target for neuroprotection and vasculoprotection via inhibition with rapamycin [29] such as the inhibition of mTOR with rapamycin preventing ANG II-mediated endothelial vascular dysfunction [31].

Unfortunately, the use of rapamycin as a drug does not come without side effects. Dyslipidaemia is a common and concerning side effect of mTOR inhibition and is observed in 40-75% of patients treated with rapamycin or rapalogs [32, 33]. Inhibition of mTORC1, seen in acute rapamycin treatment, leads to an increase in LDL cholesterol levels, hyperlipidaemia, and increased lipophagy. Chronic rapamycin treatment promoting mTORC2 inhibition also increases lipolysis via an unknown mechanism [33]. Interestingly, despite the high occurrence of dyslipidaemia, rapamycin treatment can be used to combat atherosclerosis, the process by which damage to blood vessel walls leads to blockages and therefore coronary problems such as stroke and heart attack. mTOR inhibition by rapamycin can lead to decreased atherosclerosis and potentially even reversal of disease progression [32]. The combination of rapamycin or rapalogs with stents has also been shown to be successful either as a drug eluting stent [34] or as an oral treatment alongside a base metal stent [35].

Intense research has led to the identification of potent ATP-analog mTOR inhibitors. Developed by AstraZeneca, the ATP-competitive mTOR inhibitors Torin1 [36] and Torin2 [37] can inhibit both mTORC1 and mTORC2 [25] (Figure 1A). Torin1 has been used in a few settings to demonstrate anti-aging properties. Interestingly, Torin1 was shown to be more potent than rapamycin in inhibiting senescent morphology in human cells, suggesting that these processes may rely on rapamycin-insensitive aspects of TOR signaling [38, 39]. Torin1-fed fruit flies exhibited increased lifespan without reduced fertility [40]. Regarding

treatment of age-related diseases with ATP-competitive TOR inhibitors there is currently limited data available. Nevertheless, Torin1 has no additional beneficial effects in cancer treatment compared to rapamycin: strong pan-TOR inhibition causes severe cell growth arrest and inhibition of pivotal cellular processes within all, healthy or not cells [25, 41]. Having only been synthesised in 2009, research into the effects of Torin1 and 2 on aging and age-related diseases is still in its early steps. The potency and selectivity of their action encourage further analysis of how they could be utilised in aging and pathologies.

Resveratrol

In the context of biogerontology, resveratrol is one of the most widely studied molecules [42] due its notable ability to counteract different age-related diseases in mammals with apparent lack of toxicity [43]. This polyphenolic natural product is not recommended during pregnancy, even though it is not toxic for animals [44]. It was initially described in 1939 in ethanol extracts of the white hellebore *Veratrum grandiflorum* and firstly characterized as a phytoalexin [42]. In recent years, it has been shown to be present in grapes, red wine and a few other species under stress conditions [45].

There are two isomeric configurations of this compound (3,5,4'-trihydroxystilbene) named *trans*-(E) and *cis*-(Z), which may undergo isomerization upon exposure to ultraviolet radiation [46]. The 4-hydroxystilbene skeleton in resveratrol acts as an antioxidant pharmacophore with the ability to scavenge free radicals [47, 48]. Several studies focused on this antioxidant capacity showed that it inhibits both the formation of copper-catalyzed LDL oxidation [49], and the peroxidation of membrane lipids [50]. Additionally, it restricts the release of inflammatory mediators contributing to cardiovascular disease [51], prevents the formation and growth of multiple types of cancers even by topical application in a model of skin cancer [43, 52] and is neuroprotective [53]. In contrast, a few studies have reported negative effects, such as increased atherosclerosis [54] and DNA damage [55].

Most of the health-enhancing properties of resveratrol are mediated by inhibition of cyclooxygenases enzymes COX-1 and COX-2 [56], various cytochrome P450s [57], NADPH oxidase (the enzyme responsible for reactive oxygen species (ROS) production) [58], PKD (protein kinase D) [59], S6 kinase [60], the transcription factor AP-1 (activator protein 1), and activation of sirtuins (Figure 1A), an evolutionary conserved family of NAD^+-dependent (class III) histone/protein deacetylases with a wide range of biological functions [61]. Sirtuins deacetylate a plethora of substrates such as NF-κB, p53, PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1α), FOXOs (forkhead box transcription factors) [62]. All these regulators influence the mitochondrial environment and metabolic diseases [63], and are actively involved in inflammation, carcinogenesis, lipolysis and other pathologies [64-66].

Besides the antioxidant effects of resveratrol described in humans, its ability to increase lifespan have been shown *in vitro* and *in vivo* in several eukaryotic model organisms [67]; in budding yeast this phenotype is thought to be mediated by overexpression of the enzyme Sir2 [68-70], which consumes NAD^+ resembling lifespan extension by caloric restriction [71]. Homologs of this enzyme upregulated by resveratrol in worms [72], fruit flies [73], short-lived fishes [74-76], obese mice [43], and rhesus monkeys fed a high-fat/high-sucrose diet [77] are also reported to extend lifespan. Nevertheless, relevant experiments in worms and *Drosophila* have cast doubt on the robustness of the previously reported effects of sirtuins on lifespan control [78]. Mammalian SIRT1 is the most characterized of the seven sirtuins (SIRT1-7) and it regulates various cellular processes through the correct activation of AMPK and inhibition of mTOR signaling. SIRT1 is the main target of resveratrol *in vivo* (Figure 1A) through a direct allosteric binding that enhances deacetylation of non-tagged peptide substrates [70, 79-81]. Its overexpression is beneficial in cellular models of Alzheimer's disease [82], cancer [83], type II diabetes [84], and cardiovascular disease [66]. Despite all these positive data, healthy wild-type mice show no increase in longevity by resveratrol treatment, and there are no large-scale studies on lifespan of healthy humans to date [42, 62]. Therefore, while data from the effects on mammalian lifespan in population studies is limited, it shows a remarkable capacity to enhance health and longevity in the presence of pathologies due to its pleiotropic effects, which exert physiological defence mechanisms that increase survival and make its pharmacological assessment complicated at the same time [85]. Nonetheless, it remains inconclusive whether resveratrol contains life-prolonging properties [86]. The large amount of on-going clinical trials in different pathological contexts and clinical settings is likely to produce valuable data of its effects on human health.

Targeting the GH/IGF axis in aging and age-related diseases

Growth hormone (GH) or somatotropin secretion leads to a plethora of effects connected to cellular and organismal growth and is reduced during aging, a phenomenon known as somatopause [87]. In addition, mutations in the GH axis increase lifespan, while expression of bovine GH in mice reduces it [88]. These findings resulted in proposing and even misusing human GH as an anti-aging therapy (Figure 1A). The effects of such therapies are questionable: revisiting and analysis of clinical trials on GH supplementation in the elderly revealed minor benefits related to adipose tissue composition and an increased risk of insulin resistance development or diabetes mellitus in more than half of the male participants during 26 weeks of GH therapy [89, 90]. While systemic administration of GH is probably detrimental or even dangerous, local treatments might have beneficial effects: GH injections in the knee of older individuals showed improvements in tendon collagen synthesis [91].

An interesting prospect in treatment of aging-related diseases is the utilisation of GH antagonists or somatostatin analogues (Figure 1A) normally used in treatment of acromegaly. Acromegaly, caused by excess GH in humans, is manifested by increased growth of hands, feet, face and megalocardia [92]. Patients with acromegaly are often insulin-resistant, develop diabetes mellitus, have cardiovascular problems and have increased risk of colon cancer occurrence [93]. Treatment with somatostatin analogues that inhibit GH secretion or the GH antagonist pegvisomant (that inhibits endogenous GH binding to its GH receptor), effectively brings mortality rates of acromegaly patients to those of unaffected population [93]. Pegvisomant is more efficient compared to somatostatin analogues for normalising IGF-1 levels and, while the results of combinatorial therapies are very encouraging [94], a large a global safety surveillance study (ACROSTUDY) set in 14 countries (373 sites) has concluded that pegvisomant is safe and effective for patients with acromegaly as monotherapy [95].

IGF-1/insulin signaling is extensively studied in the context of lifespan in invertebrates, with a number of studies in murine models [96]. Notably, a recent study in mice showed that insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibodies (mAb) are a promising anti-aging approach (Figure 1A): IGF-1R mAb preferentially improved female healthspan and increased median female lifespan by 9%. These changes were accompanied by a reduction in both cancer occurrence and general inflammation [97]. The best evidence of improved human healthspan for the IGF-1 pathway derives from studies on centenarians. These individuals are more likely to have IGF-1 receptor variants that are associated with reduced function. In addition, reduced IGF-1 levels are predictive of enhanced survival in female nonagenarians (i.e. women 90-99 years of age) [98].

Metformin

Metformin is the most widely used oral hypoglycemic agent and a first-line treatment for non-insulin-dependent (type 2) diabetes [99]. This compound is derived from a natural product called galegine obtained from the plant French lilac *Galega officinalis* [100]. Extracts of this plant were used in herbal medicine in medieval times for the treatment of painful/frequent urination accompanying diabetes mellitus. The glucose-lowering activity of galegine was discovered in 1920s, proving to be toxic in humans, but was re-introduced to clinical use in 1957 when was established as a safe and effective therapy for the treatment of type 2 diabetes [101]. Considering that this drug was not designed to target a specific pathway or disease and, despite its clinical use for over 60 years, the primary molecular mechanism of this dimethylbiguanide has remained unclear [99]. Several studies have established that the major effects of the compound are the suppression of >60% gluconeogenesis/lipogenesis in the liver and the increase in insulin-mediated uptake of glucose in muscles [102, 103]. These have been attributed to different pathways including AMPK (Figure 1A).

The adenosine monophosphate-activated protein kinase (AMPK) pathway is an evolutionarily conserved signaling pathway that senses cellular energy status through AMP/ATP and ADP/ATP ratios promoting catabolism and inhibiting anabolism [104]. It is not a surprise that dietary restriction activates AMPK and nutritional or caloric overload inhibits it, leading to increased occurrence of metabolic syndrome [105, 106]. AMPK modulates multiple processes such as gluconeogenesis, lipid oxidation, mitophagy and protein synthesis through TOR regulation [104]. AMP-activated protein serine/threonine kinases are heretotrimeric consisting of one enzymatic and two regulatory subunits. AMPK is tightly regulated by upstream kinases and phosphatases such as liver kinase B (LKB), Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) and transforming growth factor-β-activated kinase 1 (TAK1) [107-109]. Intense studies have provided insights on the structure and function of AMPK and how

small molecules can inhibit or enhance its actions [110, 111].

AMPK is directly linked to aging. Beyond cellular energy status AMPK coordinates and controls repair and housekeeping mechanisms linked with maintenance, senescence and lifespan, such as autophagocytosis [112], ER stress suppression [113], oxidative stress alleviation [114] or suppression of inflammation [115]. Overexpression of AMPK can extend lifespan in worms [116] and *Drosophila* [117]. In mammals the situation is complicated, with gene deletions having severely detrimental effects [118]. AMPK activation through physical exercise has beneficial effects [119], while activation in pathological conditions such as stroke can enhance pathologies and induce additional cellular and tissue damage [120, 121]. The ability of AMPK to be activated upon certain stimuli declines during lifespan. For example, AMPK activity increases within muscles of young but not old rats [122]. Decline in the AMPK activation ability with aging is now linked with age-related diseases such as cardiovascular diseases and metabolic syndrome [123, 124]. As already mentioned, physiological regulation of AMPK through nutrition and exercise is possible. Nevertheless, increasing data strongly suggests potent activation of the pathway with the use of drugs, some of them as common as metformin and aspirin.

Metformin-mediated actions that are AMPK-dependent involve phosphorylation/activation of AMPK in its catalytic α1/α2 subunits at threorine-172, mediated by LKB1 (liver kinase B1) [125]. This is triggered by the inhibition of the mitochondrial respiratory chain complex I, which produces a decrease in ATP levels together with an increase in the ratios of ADP/ATP [126] and AMP/ATP that activate AMPK [127]. The latter results leading in subsequent phosphorylation of CRTC2 (CREB-regulated transcription coactivator 2) and CBP (CREB-binding protein), that downregulate gluconeogenic gene expression [128, 129]. Interestingly, another study reported an AMPK-independent pathway based on results from AMPK α1/α2 knockout mice, indicating that the decrease in glucose production occurs through the regulation of gluconeogenesis flux in response to a decrease of the hepatic energy availability instead of direct suppression of gluconeogenic gene expression [130].

Lifespan extension is among the therapeutic opportunities being explored for metformin. They have been attributed to transcription, genome stability, epigenome marking, metal-interactive regulation of protein function that inhibit pro-inflammatory proteases and anti-apoptotic pathways independent of its glucose reducing effects [131-134]. This was proved in various systems including *S. cerevisiae* where extension of chronological lifespan was reported due to glycation inhibition (mimics calorie restriction) restoring deregulated proteins involved in mitochondrial respiration and facilitating the shift of metabolism from fermentation to respiration [135]. In *C. elegans* metformin works via impairment of folate and methionine metabolism in the intestinal microbiome [136], while in mice reduces adipose tissue inflammation [137]. Within fly intestinal stem cells, metformin inhibits aging phenotypes in an Atg6 (autophagy-related 6)-dependent manner [138]. Additionally, metformin is reported to inhibit differentiation of monocytes into macrophages through disruption of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling [139]. In human plasma it suppresses proinflammatory cytokines such as CCL11 (CC motif chemokine 11) involved in age-related cellular and tissue dysfunction and affects the neutrophil to lymphocyte ratio, which is a marker of inflammation linked to mortality [140].

Clinical trials in humans confirmed the significant reduction of CRP (C-reactive protein) levels, which is a marker of systemic inflammation. Interestingly, such an effect is not observed with resveratrol treatment [99]. In cells derived from patients with Hutchinson-Gilford progeria syndrome, metformin lowers progerin production (a toxic protein also present in normal subjects at lower levels). This is done through inhibition of SRSF1 (serine-arginine rich splicing factor 1) alleviating pathological defects. In this scenario, cellular stress-induced AMPK activation promotes both the activity of the splicing factor-associated protein p32. This protein acts as endogenous inhibitor of SRSF1 and binds to mitochondrial mRNAs *in vivo*, regulating their efficient translation. As these RNAs code for proteins involved in oxidative phosphorylation, p32 is pivotal in maintaining this essential cellular function. In addition, p32 affects the transcriptional activity of NFE2L2 (Nuclear factor erythroid-derived 2-like 2), a main regulator of the antioxidant response disrupted in progeria cells [134]. Another possibility to explain the health span-extension effects of metformin is through the inhibition of nutrient/energy-sensing metabolic pathways such as the insulin/IGF-1 (insulin-like growth factor 1) and mTOR [133, 141]. Other studies have reported neuroprotective effects against cognitive dysfunction by hippocampal neurogenesis/differentiation and inhibition of aging-related neuroinflammation in mice [142-144]. The overall favourable effects of metformin in physiological functions through multiple modest, but substantial, effects, combined with its well-characterized profile, suggest that it may be beneficial for the treatment of normal or pathological aging [134].

Aspirin

Acetylsalicylic acid is the most common nonsteroidal anti-inflammatory drug and globally used medication. Considered against its potential side effects of producing stomach ulcers and bleeding, is currently widely used for the treatment of pain, fever, inflammation, platelet aggregation, prevention of cardiovascular pathologies and cancer [145]. Its active component salicylate is chemically synthesized from the bark of the willow tree *Salix alba*. Its therapeutic use dates back to ancient times but pure acetyl salicylate has been manufactured and commercialized since 1899 [146, 147].

Aspirin affects multiple signal transduction pathways; the principal mode of action discovered in 1971 is through the irreversible inactivation of COX-1 (prostaglandin-endoperoxide synthase, PTGS1) and the inhibition of COX-2 (PTGS2), suppressing prostanoid biosynthesis [148] (Figure 1B). The drug is rapidly broken down *in vivo* to salicylate. The latter has multiple effects such as uncoupling oxidative respiration through proton transport on the inner mitochondrial membrane [149]; acetylation and inhibition of G6PD (glucose-6-phosphate dehydrogenase), which catalyzes the first reaction in the pentose phosphate pathway involved in the regulation of oxidative stress [150]; activation of AMPK via Thr-172 phosphorylation; and activation of protein kinase IKKβ (inhibitor of nuclear factor kappa-B) that arrests the pro-inflammatory transcription factor NF-κB [151]. Additionally, salicylate competitively inhibits the binding of acetyl coenzyme A (the sole acetyl group donor) to acetyltransferases such as EP300 (E1A-associated protein p300) thus inhibiting its activity and inducing the autophagic cascade that enhances longevity, still observed in the absence of AMPK [152, 153].

The pro-health benefits of aspirin also include the delay in the onset of various age-related diseases and an increase in the maximum and mean lifespan of different organisms through pleiotropic molecular mechanisms [154]. Lifespan extension have been reported in worms via attenuation of endogenous levels of ROS as well as upregulation of antioxidant genes [155], activation of the transcription factors DAF-12 and DAF-16 that increase lipid hydrolysis and inhibit the proliferation of germline stem cells without alterations in the number of offspring [154]. In the fruit fly, it has been associated to a decrease in female fecundity produced by the inhibition of the heme peroxidase Pxt, a COX-like facilitator of follicle maturation [156]. These effects are accompanied by increased resistance to stress and improved locomotor activity that are overall mediated by the Pkh2-ypk1-lem3-tat2 signaling pathway [157]. Interestingly, in mice, aspirin increases the survival of males but not females [158]. Different trials have shown protective effects against Alzheimer's and Parkinson's disease, low risk of cancer incidence and mortality[159, 160] with metabolic and immunity functions [154]. However, the successful use of aspirin in humans to actually increase healthspan and lifespan has yet to be tested [161].

Future prospects

The current challenges for the treatment of multiple age-related diseases are focused on the identification of molecules that could increase human lifespan and the development of strategies to safely assess them in the population. The common mechanisms of action observed between aspirin, resveratrol and metformin could bring light to this matter by pharmacologically inducing the benefits of caloric restriction. Statins are inhibitors of the HMG-CoA-competitive reductase and exhibit a plethora of effects. They affect cholesterol production and protein prenylation resulting in improvement of endothelial and immune function as well as in other cardiovascular benefits [162]. Despite the heterogeneity and inconclusive results from clinical trials, statins present some potential in terms of their anti-inflammatory properties and in the possibility of enhancing or improving present therapies for some types of cancer [162].

Angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II receptor blockers are used for hypertension treatment. Nevertheless, Angiotensin II is shown to be implicated in age-related cardiovascular disease in rats [163]. The renin-angiotensin system is shown to play an important role in aging kidneys [164], while angiotensin II inhibitors increase the lifespan of hypertensive rats [165, 166]. In humans, there is no large study for the effects on lifespan and aging rates. However, beneficial effects on cardiac hypertrophy [163] and lower incidence of cancer [167] are very promising.

Recent data has shown that the Ras-Erk-ETS pathway is a drug target of longevity [168] with trametinib, a specific inhibitor of the Ras pathway being able to extend lifespan via adult-onset administration [168] in the fruitfly. The ETS transcriptional repressor, Anterior open (Aop) is central to the lifespan extension downstream of reduced insulin/IGF-1 signaling or Ras attenuation [168]. The Ras pathway is highly conserved and these data show promise for anti-aging targets in humans. Finally, inhibition of the TORC1-regulated RNA Polymerase III, an essential enzyme responsible for transcribing tRNAs and 5S ribosomal RNA,

extends lifespan in yeast, worms and fruitfly [169]. Pol III inhibition within adult worm or fly guts is sufficient to extend lifespan and results in amelioration of age-related gut deterioration and pathology. These results, place Pol III as an interesting potential target for pharmacological interventions.

Dietary restriction, activation of AMPK through metformin and inhibition of TOR through rapamycin and Torins lead to increase in autophagy. Autophagy is a degradation pathway leading to recycling of cellular material and removal of damaged macromolecules and organelles that may pose a burden to the cell. Autophagy plays a crucial role in cellular homeostasis, development, immunity, tumour suppression and cellular metabolism, prevention of neurodegeneration and lifespan extension. Therefore, pharmacological stimulation of autophagy may be an effective approach for preventing or ameliorating certain human diseases and reducing aging symptoms. Towards identifying new autophagy inducers, high-throughput screens with chemical compound libraries containing around 300,000 compounds are utilised. In a recent screen three candidate molecules have been identified that may be clinically useful as autophagy-inducing agents [170]. Interestingly, a cell-based quantitative high-throughput image screening (qHTS) for autophagy modulators using mouse embryonic fibroblasts (MEFs) has identified (apart from a number of novel autophagy inducers, inhibitors, and modulators) a group of compounds related to dopamine receptors. These compounds are commonly used as clinical psychiatric drugs. These include indatraline hydrochloride (IND), a dopamine inhibitor, and chlorpromazine hydrochloride (CPZ) and fluphenazine dihydrochloride (FPZ), two dopamine receptor antagonists. FPZ-induced autophagy happens through mTOR inhibition but IND and CPZ can induce autophagy via a TOR-independent manner [171]. These results underline once again the importance of revisiting and repurposing already tested and trialled drugs in biogerontology. Further studies on the same theme focus on natural products [172].

While intense biogerontology research is focused on genetic factors it is now evident that epigenetic regulators impact greatly on lifespan. Increasing evidence shows that histone deacetylase (HDAC) inhibitors, able to reverse the deacetylation of histone tails and activate the expression of particular genes, are a promising class of anti-aging compounds that can play major roles for combating age-related diseases [173]. HDAC inhibitors have lifespan-extending effects to preclinical animal models such as fruitflies, *C. elegans* and rodents [173]. Importantly, preclinical and clinical studies using HDAC inhibitors for age-related conditions have generated very positive outcomes: a wide range of these molecules have emerged as anticancer drugs [174]. A number of them are already approved for specific lymphomas and haematological cancers while others are currently on different stages of clinical development and trials [175, 176]. HDAC inhibitors have also been shown to be beneficial in the contexts of neurodegenerative disease, metabolic and cardiovascular problems as well as inflammatory conditions [173].

Organismal aging has a cellular component and can be viewed as the combination of limited chronological and replicative lifespan of the cells that the organism is composed of. While chronological lifespan is defined as the time that a postmitotic population is viable, replicative lifespan is the number of mitotic divisions that a mother cell can give rise to until senescence. Cellular senescence, first described by Hayflick and Moorhead in the 1960s, is directly linked with decreased telomere length and decreased telomerase activity. During aging and exposure to various intra- and extracellular stresses a rise in senescent cells is observed. If senescent cells are not removed, the senescence state is induced in neighbouring young cells leading to tissue dysfunction [177]. Telomerase activator therapies are currently undergoing with at least one product, TA-65, already available. TA-65 is able to increase telomerase levels and healthspan in mice. Nevertheless, mean and maximal lifespan was not increased [178]. TA-65 has also been reported to decrease senescent immune system cells in patients [179]. Telomerase-based interventions although not fully understood will have no effects on the chronological lifespan of cells within the body (such as postmitotic brain cells). In addition, as telomerase favours tumorigenesis, its long-term efficiency and safety as anti-aging therapy is still questionable [177].

An area of intense interest in drugs with rejuvenating potential related to senescent cells is senolytics. Senolytic drugs (term originating from 'senescence' and 'lysis') selectively destroy senescent cells from tissues leading to improved health markers in mouse models. The first molecules to be identified as senolytics in 2015 were Dasatinib and Quercetin. Dasatinib, a small molecule targeting BCR/Abl (the Philadelphia chromosome), Src, c-Kit, ephrin receptors, and several other tyrosine kinases. Dasatinib is sold under the brand name Sprycel and is a chemotherapy medication used to treat certain cases of chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). Quercetin is a plant flavonol from the group of polyphenols and is found in fruit, vegetables, leaves, and grains. Dasatinib is able to eliminate senescent human fat cell progenitors, while

Table 1: Drugs discussed in the manuscript, their effects on the lifespan of animal aging models and clinical studies in humans related aging and aging phenotype. The effects of somatostatin and pegvisomant on animal aging rates, median and maximal lifespans is unclear. The bar sign (–) within the clinical studies column of the table designates that there are no studies for direct effect on human lifespan to date (data from clinicaltrials.gov as of 30.11.18).

Drug	Longevity effects on animal models	Clinical Trials for aging or age-related disease[1]	
		Total	Completed
Metformin	**Yes, positive**	23	7
Rapamycin	**Yes, positive**	12	3
Torin1	**Yes, positive**	0	-
Resveratrol	**Mixed results**	16	10
Somatostatin	**-**	2	2
Pegvisomant	**-**	0	-
Aspirin	**Yes, positive**	8	5

quercetin is more effective against senescent human endothelial cells. The combination of Dasatinib and Quercetin is effective in eliminating senescent mouse embryonic fibroblasts. Combinations of Dasatinib and Quercetin are shown to reduce senescent cells in aged, and progeroid mice [180]. Navitoclax (ABT-263) was the third senolytic to be identified [181]. Navitoclax is a protein–protein interaction inhibitor targeting the BCL-2 family of apoptotic proteins. The finding that BCL-2 inhibitors can act as senolytics helped towards the identification of analogues A1331852, A1155463 and ABT-737 that inhibit BCL-2 family members, as senolytics [182]. In addition, the action of the flavonol fisetin and the alkaloid piperlongumine as senolytics or senotherapeutics is now established [182-184]. A FOXO4 peptide able to perturb the FOXO4 interaction with p53 was identified [185]. In senescent cells, this selectively causes p53 nuclear exclusion and cell-intrinsic apoptosis. Under conditions where it was well tolerated *in vivo*, this FOXO4 peptide neutralized doxorubicin-induced chemotoxicity. Moreover, it restored fitness, fur density, and renal function in both fast aging and naturally aged mice [185]. This is an exciting focus in biogerontology studies with new data amounting on the beneficial effects of senolytics. Nevertheless, their effects on young tissues are not quite clear yet [186]. A characteristic of this research is again the repurposing of known and trialled drugs.

It is more than evident that the field rapidly progresses through the identification of lifespan-increasing genetic and environmental factors. However, the need now is the identification of those interventions that increase human healthspan while not enhancing morbidity periods through clinical trials. Focused drug design studies as well as pre-clinical and clinical trials are of essence to realise the treatments that could delay and ameliorate or even prevent age-related ailments (Table 1).

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