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The Role of Rapamycin in Healthspan Extension via the Delay of Organ Aging

Running title: The role of rapamycin in extending healthspan

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Highlights:

- Organ aging may lead to a variety of diseases, which is not beneficial to a healthy lifespan.
- Rapamycin can prolong the lifespan of many species by inhibiting mTOR.
- The aging of multiple systems and organs could potentially be alleviated by treatment with rapamycin, mainly via blocking mTOR signaling.

Abstract

Aging can not only shorten a healthy lifespan, but can also lead to multi-organ dysfunction and failure. Anti-aging is a complex and worldwide conundrum for eliminating the various pathologies of senility. The past decade has seen great progress in the understanding of the aging-associated signaling pathways and their application for developing anti-aging approaches. Currently, some drugs can improve quality of life. The activation of mammalian target of rapamycin (mTOR) signaling is one of the core and detrimental mechanisms related to aging; rapamycin can reduce the rate of aging, improve age-related diseases by inhibiting the mTOR pathway, and prolong lifespan and healthspan effectively. However, the current evidence for rapamycin in lifespan extension and organ aging is fragmented and scattered. In this review, we summarize the efficacy and safety of rapamycin in prolonging a healthy lifespan by systematically alleviating aging in multiple organ systems, i.e., the nervous, urinary,

digestive, circulatory, motor, respiratory, endocrine, reproductive, integumentary and immune systems, to provide a theoretical basis for the future clinical application of rapamycin in anti-aging.

Keywords: Rapamycin, Healthspan, Organ aging, mTOR signaling, Adverse effects

1. Introduction

Aging has been defined as the late stage of biological and physiological processes. It refers to all organs confronting the fate of functional decline and ultimate tendency to death. This decay process is often accompanied by the emergence of age-related diseases, such as neurodegeneration (Parkinson disease and Alzheimer disease), atherosclerosis, type 2 diabetes, osteoporosis, and cardiovascular disease (López-Otín et al., 2013; Tchkonia and Kirkland, 2018). An increasing number of countries are proceeding towards an astogeny (colony-reducing) society along with the need for life quality, and aging has a profound influence on the economy, society, and individual health. Hence, there should be sufficient focus on pursuing the possibility of extending lifespan. Probing and understanding the mechanism of senility enables the identification of underlying methods to withstand aging. Currently, genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication are the common working mechanisms of aging (López-Otín et al., 2013). Moreover, many genetic pathways regulate vertebrate aging, such as that for insulin/insulin-like growth factor (IGF), mammalian target of rapamycin (mTOR), and adenosine monophosphate-activated protein kinase (AMPK) (Singh et al., 2019b). Although the aging process cannot be halted, it is possible to slow it via lifespan extension and promote healthy aging. “Healthy aging” is a new concept that defines age as being healthy across multiple physiological systems simultaneously. This concept is absent from life-affecting diseases, and refers to good quality of life during aging, including freedom from major illness and disability, and high physical and cognitive functioning, opening a new chapter on aging research (Hansen and Kennedy, 2016; McLaughlin et al., 2012; Wong, 2018). In attempts to improve healthspan or increasing health aging, scientists have explored various pharmacological agents potentially helpful to this endeavor since the 1930s, when biologists first reported calorie restriction as an approach to enhancing lifespan in mice and rats (Campisi et al., 2019).

Pharmaceutical methods are now considered the main means of delaying aging. Over the past decades, various anti-aging drugs, such as the mTOR inhibitor rapamycin; antioxidants such as resveratrol, melatonin, and coenzyme Q10 (CoQ10); and especially senolytics such as dasatinib and quercetin (D+Q) or fisetin, have shown promising effects on longevity by targeting mTOR, mitochondrial and oxidative stress, and cellular senescence, respectively (Campisi et al., 2019; Gorgoulis et al., 2019; Hargreaves and Mantle, 2019; Ramis et al., 2015; Wilkinson et al., 2012; Xu et al., 2018; Yousefzadeh et al., 2018; Zhu et al., 2015). Among them, rapamycin stands out as a strong anti-aging candidate. However, it is unknown whether rapamycin can extend

lifespan to a consistent extent on all animal platforms, and whether it has a comprehensive effect on mitigating age-related diseases in various organs, achieving healthy aging in humans.

In the present review, we systematically present the maximum lifespan extension of model animals at different levels of evolution. Given the complexity of the issue, we have attempted to systematically identify and categorize the effect of rapamycin on alleviating the aging of multiple organs to characterize pioneer insights into 10 human body systems: (i) nervous; (ii) reproductive; (iii) urinary; (iv) digestive; (v) circulatory; (vi) musculoskeletal; (vii) respiratory; (viii) endocrine; (ix) integumentary; and (x) immune. Finally, we discuss the emerging clinical and scientific toxicities and adverse effects of rapamycin, aiming to provide comprehensive information to transform the development of a conventional drug to new use for advancing human health with greater safety.

2. The origin of rapamycin and its role in lifespan extension

2.1 The origin of rapamycin

Rapamycin was identified in 1975, initially isolated as an antibiotic by Sehgal et al. in soil from the South Pacific island of Rapa Nui, where the islanders never wore shoes but were rarely infected with tetanus (Sehgal et al., 1975; Vezina et al., 1975). It became known as a macrolide antifungal agent. Subsequently, in addition to its anti-inflammatory effect, it was found that rapamycin also suppresses the immune system, and it was approved as an oral organ transplant immune inhibitor in 1999. Nowadays, rapamycin is clinically used for suppressing tissue rejection after organ transplantation and as adjuvant therapy for some cancers (Mossmann et al., 2018). In 2009, it was discovered that rapamycin extends the maximal lifespan of both male and female mice by 9% and 14%, respectively (Harrison et al., 2009).

Since then, research on the role on rapamycin in anti-aging has increased tremendously. Some mouse studies have demonstrated its powerful pharmacological effect on delaying aging, extending healthspan, and attenuating age-related diseases (Anisimov et al., 2010; Zhang et al., 2014) by inducing autophagy, as this cellular process is related to senescence. Accumulating senescent cellular molecules might trigger autophagosomes to remove “trash” molecules and to maintain self-homeostasis (Madeo et al., 2015). In mice, rapamycin activates autophagy and promotes lifespan effectively (Fernandez et al., 2018). Amazingly, although there were several discomforts during rapamycin treatment in humans, no obvious changes in health status were observed (Kraig et al., 2018). These studies suggest that rapamycin could be an ideal anti-aging supplement. Accordingly, we discuss in this review the recent advancements in the impact of rapamycin on improving the health status of each organ system and provide a perspective for its potential application for extending the human healthspan.

2.2 The role of rapamycin in lifespan extension

Rapamycin increases the lifespan of multiple species by inhibiting mTOR. Some papers have discussed the detailed mechanisms for the anti-aging benefit of rapamycin, as an mTOR inhibitor, in different species. mTOR is a highly conserved serine/threonine kinase of the phosphatidylinositol kinase-related kinase family that plays a central role in sensing and responding to nutrient availability and growth signaling in eukaryotes, and consists of two complexes: mTORC1 and mTORC2. Due to its essential value in modulating growth factor sensing, insulin/IGF/phosphoinositide-3-kinase (PI3K)/AKT, ribosome capacity, AMPK, lysosomal amino acid sensing, caloric restriction, mRNA translation, autophagy, mitochondrial function, and metabolism and stem cell maintenance, mTOR plays a critical role in aging (Barja, 2019; Ehninger et al., 2014; Johnson et al., 2015; Kapahi et al., 2010). There is support for the premise that inhibiting mTOR can extend lifespan by reducing mRNA translation and protein synthesis by activating ribosomal S6K1 (S6 protein kinase) and inhibiting 4E-BP1 (eukaryotic translation factor 4E-binding protein 1), and further genetic research shows that that *S6k1* knockout mice had increased maximal lifespan and resistance to age-associated traits, proving this thesis (Selman et al., 2009; Sharp and Strong, 2010). Besides, an mTOR(Δ/Δ) hypomorphic mouse model expressing 25% of the mTOR levels as compared to wild-type mice not only had increased lifespan, but also enjoyed some benefits in tissue and organ aging (Wu et al., 2013). These findings intensively implicate mTOR in mediating mammalian longevity.

There is mounting evidence that rapamycin-induced mTOR inhibition prolongs the lifespan of many species, ranging from nematodes to rodents. Alvers et al. (2009) showed that the administration of rapamycin in yeasts activated autophagy and regulated amino acid homeostasis to increase the *Saccharomyces cerevisiae* chronological lifespan by decreasing mTOR activity (Alvers et al., 2009). The lifespan extension following rapamycin treatment in chronologically aged *S. cerevisiae* was dose-dependent (2.9–4.51 at 1 ng/mL) (Powers et al., 2006). In nematode models, rapamycin treatment resulted in longer lifespans, and even had equal efficacy in extending the lifespans of long- and short-lived lines by downregulating mTOR signaling (Lind et al., 2017; Lind et al., 2016; Robida-Stubbs et al., 2012). In rotifers, rapamycin (1 μM) extended the mean and maximum lifespan by 35% and 37%, respectively (Snell et al., 2014). In *Drosophila*, rapamycin (0.005 μM) increased the lifespan without decreasing the quality of life, significantly increasing the median lifespan by 14% and 12% in males and females, respectively (Bjedov et al., 2010; Danilov et al., 2013). Further, rapamycin lengthened the maximum lifespan in *Drosophila* by 14.5% (Wang et al., 2016). In mammal model species, rapamycin extended the maximal lifespan of both male and female mice by 9% and 14%, respectively (Harrison et al., 2009). Neff et al. reported the lifespan-extending effect of rapamycin in C57BL/6J mice, and different age of treatment onset in young adulthood (4 months old), midlife (13 months old), or late in life (20–22 months old) rescued some aging traits (Neff et al., 2013). The longest lifespan extension by rapamycin was 26% (Miller et al., 2014) (**Fig. 1**).

To date, the longevity-increasing effects of rapamycin have been observed in several species, namely yeasts, nematodes, *Drosophila*, and mice (Johnson et al., 2013). There has been no study of rapamycin in the lifespan extension of humans and primates until now, and exploration thereof is necessary. In addition, along with the lifespan extension by rapamycin treatment, more attention should be focused on whether there is delayed aging of organs from various systems. The following text focuses on the effect of rapamycin on multi-organ aging.

3. Rapamycin and age-related diseases

Organ function declines during aging, evolving into the subsequent emergence of organ aging or age-related disease. As rapamycin might result in longer lifespans, we wondered if organ function or age-related disease recovery or mitigation, which represent a longer healthspan, could appear as well. The present review presents systematic conclusions on informed research on the role of rapamycin in organ aging and age-related disease, and discusses the potential of rapamycin in extending healthspan by delaying the aging of multiple organs systematically.

3.1 Nervous system

3.1.1 Age-related neurodegenerative disorders

The nervous system plays a dominant role in commanding the physiological functions. During nervous system aging, structural and functional alterations increase the risk of neurological diseases, considered the biggest research hotspot in aging. Parkinson and Alzheimer disease are the most common age-related neurodegenerative disorders, with the characteristics of insidious onset and progressive development. Both diseases cause serious cognitive problems. The symptom of Alzheimer disease is the complete impairment of cognitive function, accompanied by extracellular deposition of amyloid-beta (Aβ) peptide, the biomarker of the disease. By promoting autophagy, rapamycin (0.5 mg/kg bodyweight [bw] for 28 days) conferred significant protection against aging-related oxidative stress, cell death, neuroinflammation, and neurodegeneration in the brains of old rats (Singh et al., 2019a). Cognitive impairment is greatly prevalent in aging, and has high relevance to mTOR (Van Skike et al., 2020). Intraperitoneal rapamycin (0.25 mg/kg bw for 3 weeks) induced autophagy and activated PI3K–Akt1–mTOR–CREB signaling to reverse Aβ-induced oxidative stress, synaptic dysfunction, and neurodegeneration in adult rat hippocampus (Singh et al., 2017a). CREB is a neurogenesis-related transcription molecule that binds to brain cells and acts by stimulating certain genes to strengthen the bonds between cells and to regulate neuronal plasticity and long-term memory formation in the brain (Shi et al., 2020; Yin et al., 1995). With PI3K, Akt1, and mTOR, it forms a well-known pro-survival signaling pathway implicated in neuronal cell survival. These studies suggest that rapamycin can target PI3K–Akt1–mTOR–CREB signaling to alleviate Alzheimer disease.

A definite neurodegenerative disease, the clinical signs of Parkinson disease are

static tremor, bradykinesia, and myotonia; its exact etiology and pathogenesis remain elusive. Lysosomal autophagy for degrading damaged mitochondria is now considered the leading pathway for maintaining mitochondrial homeostasis, the disruption of which results in Parkinson disease (Senkevich and Gan-Or, 2019). In a Parkin Q311X mutant mouse model, rapamycin (2.24 mg/kg bw starting at 13 months of age for 12 weeks) reversed the impaired mitochondrial quality control and neurodegenerative features through PARIS-mediated regulation of PGC1α–TFEB signaling (Siddiqui et al., 2015a). A recent meta-analyses of genome-wide gene expression in autopsied tissues from patients with sporadic Parkinson disease and age-matched controls noted that reducing PGC1α and its target genes, including *TFEB*, is a core feature, suggesting that this signaling pathway is a potentially important therapeutic target (Zheng et al., 2010). Rapamycin also had neuroprotective efficacy via anti-inflammation to increase interleukin-6 (IL-6) expression in MPTP mice (a Parkinson disease model) via the mTOR–Akt–NF-κB cascade (Zhang et al., 2017). In summary, rapamycin exerts protective effects against Parkinson disease by restoring PGC1α–TFEB and mTOR–Akt–NF-κB signaling.

3.1.2 Age-related macular degeneration

Age-related macular degeneration (AMD), both a vascular retinal and neurodegenerative disease, is the common cause of blindness in the elderly (Swain and McGwin, 2019). Retinal pigment epithelial (RPE) cell impairment is the main etiological component of AMD. Rapamycin reversed inflammation and decreased angiogenic factors in A2E-treated human RPE cells by magnifying autophagy levels through the mTOR pathway against A2E, a major component of toxic lipofuscin implicated in AMD deposition in RPE cells with aging (Zhang et al., 2015a). *In vivo*, rapamycin-treated accelerated-senescence OXYS rats with natural age-related retinopathy exhibited a significant decline in the incidence and severity of retinopathy, with decreased nuclei heterogeneity and regularized normal intervals between the nuclei in the RPE cell layers, and decreased nuclear and cellular pyknosis in photoreceptor cells (Kolosova et al., 2012).

Human studies have reported several direct pieces of evidence for the rapamycin-associated benefits of geographic atrophy (GA), which is the form of late AMD; however, no anatomical or functional protection in treated eyes with AMD was indicated (Gensler et al., 2018; Petrou et al., 2014; Wong et al., 2013), and it may even be potentially linked with detrimental effects on visual acuity (Wong et al., 2013). Another clinical trial on rapamycin as an immunosuppressive therapy reported that it did not alter the visual acuities of AMD patients (Nussenblatt et al., 2010).

3.1.3 Age-related hearing loss

Age-related hearing loss (ARHL) is the most prevalent form of hearing loss in humans. mTORC1 signaling in aging C57BL/6J mice is highly and specifically activated in the cochlear neurosensory epithelium (NSE), and rapamycin injection prevented ARHL by overactivating mTORC1 signaling, suggesting that reducing mTORC1 activity in cochlear hair cells may be a promising means of treating and

preventing ARHL (Fu et al., 2018). Rapamycin also delayed age-related loss of cochlear hair cells, an ARHL component, in 22-month-old male and female UM-HET3 mice (Altschuler et al., 2018).

Generally, the cognitive health of the aged is considered an important badge of healthy aging. To a certain extent, rapamycin is helpful in protecting against aging- and nervous system-related diseases.

3.2 Reproductive system

3.2.1 Ovarian aging

In females, ovarian aging is shown in the rapid decline in follicle number and quality, which affects not only ovarian health but also the other symptoms of hormone deficiency, such as cardiovascular health and osteoporosis (Li and Wang, 2018; Quinn and Cedars, 2018; Younis, 2012). mTOR signaling is broadly recognized as a leading key of signals and pathways for cellular metabolism, proliferation, and differentiation, with the ability to control numerous life processes, including ovarian development, by regulating folliculogenesis, oocyte meiotic maturation, and ovarian somatic cell growth (Guo et al., 2016; Guo and Yu, 2019). mTOR is expressed at all stages in oocytes, and can determine oocyte quality and fertility in mice with stage-specific effects in mediating sustained oocyte genomic integrity, promoting the completion of meiosis and enabling embryonic development (Guo et al., 2018). Hence, these studies suggest that mTOR may play an important part in maintaining female reproduction.

On natural ovarian aging, Garcia et al. (2019) reported that mice treated with rapamycin (4 mg/kg bw every other day) for 93 days had a higher number of primordial follicles, representing better ovarian reserve by reducing primordial follicle activation without altering body weight but inducing insulin resistance, as compared to the controls. The authors inferred that it involved higher expression of ovarian *Foxo3a*, a transcription factor of the downstream PI3K–AKT–mTOR pathway, in mouse ovaries (Garcia et al., 2019). In both 8-week-old and 8-month-old mice, short-term (2-week) administration of rapamycin prolonged ovarian lifespan, increased oocyte quality, and improved the ovarian microenvironment, with a sharp decline in phosphorylated rpS6 (p-rpS6), the mTORC1 downstream marker. However, the phosphorylation level of Akt, the classic downstream factor of PI3K, was unaltered. As p-Akt can also be activated by mTORC2, it was speculated that short-term rapamycin treatment affects neither the PI3K nor mTORC2 signaling pathways. Although the mice exhibited disrupted ovarian function during and shortly after rapamycin treatment, ovarian function eventually returned to normal 2 months later, with regular estrus cycles, normal p-rpS6 levels, ovarian weight, morphology, and serum hormone levels as compared to the control mice (Dou et al., 2017a). On premature ovarian failure (POF), rapamycin inhibited cyclophosphamide-induced PI3K–Akt–mTOR signaling pathway overactivation to prevent primordial follicle activation and protected the ovarian reserve from progressing towards POF (Zhou et al., 2017).

However, clinical single-center observational data showed that low-dose oral rapamycin appeared to increase the risk of menstrual cycle disturbances and ovarian cysts in patients with autosomal dominant polycystic kidney disease (ADPKD) (Braun

et al., 2012). This human adverse event is partly consistent with the mouse research by Dou et al., suggesting that rapamycin could potentially disrupt the menstrual cycle, but that it may eventually return to normal.

3.2.2 Age-related endometrial hyperplasia

The incidence rates of endometrial hyperplasia and endometrial cancer are markedly increased during aging (Ghoubara et al., 2018; Lacey et al., 2010). mTOR was highly activated in the hyperplastic endometrium in aged mice, and mTOR signaling overactivation resulted in endometrial hyperplasia. Rapamycin treatment inhibited endometrial hyperplasia in aged mice, suggesting that mTOR may play a key role in rapamycin therapy (Bajwa et al., 2017).

An interesting finding from a clinical trial was that, compared to other different immunosuppressive regimens that were identified to cause endometrial hyperplasia in women who had received solid-organ transplantation, no endometrial hyperplasia occurred in the rapamycin group, indicating that it may be a reliable choice for people at risk of endometrial hyperplasia (Tohma et al., 2018).

3.2.3 Age-related testicular atrophy

There are very few studies on rapamycin in male reproductive system aging. Only one study noted that rapamycin protected against age-caused testicular atrophy by increasing spermatid, spermatocyte, differentiating spermatogonia, and primary spermatogonia numbers (Wilkinson et al., 2012). Overall, these studies suggest that rapamycin has the potential for protecting healthy aging of the reproductive system.

3.3 Urinary system

3.3.1 Renal aging

Autophagy, controlled by mTOR in the kidney, has a vital role in mediating cellular metabolic and organelle homeostasis (Kaushal et al., 2020). On aged kidneys, rapamycin-treated mice (11–27 weeks old) had lower urine albumin/creatinine ratios, showing improved kidney function compared to the controls (Reifsnyder et al., 2018). Rapamycin-treated old mice (26.5 months equals 75 years in human age) had increased numbers of glomeruli with a crescentic appearance, mediated by mTOR inhibition (McNicholas et al., 2016). However, Diao et al. (2019) held a different perspective, believing that rapamycin could only reverse ischemia-reperfusion (I/R)-caused acute renal injury (AKI) in young rats (3 months old), while this protection was not present in the kidneys of aged rats (24 months old) (Diao et al., 2019). Nevertheless, another study suggested that, in aged groups, rapamycin may not be protective against ischemia- and cisplatin-induced AKI due to the failure of enhancing autophagy (Andrianova et al., 2019). In that study, the aged group had a much lower basal autophagy level as compared with young rats under the same interventions, and that may be the underlying reason for the loss of the protective effect against autophagy in aged rats. To summarize, the benefits of rapamycin on renal aging remain controversial.

Rapamycin has shown some potential for preventing renal aging, but studies have been limited to animals, and its effects require confirmation. Moreover, its role in other

urinary system organs remains to be discovered. Further and more studies should be conducted.

3.4 Digestive system

3.4.1 Age-related periodontal disease

Periodontal disease is characterized by the apparent loss of alveolar bone and connective tissue degeneration, and morbidity increases with age. The effects of inhibiting mTOR on aging periodontal tissue remain to be explored. An et al. (2020) reported that, after 8 weeks of diet containing rapamycin, 22-month-old aged mice had attenuated alveolar bone loss and increased youthful levels of alveolar bone (An et al., 2017). The same team subsequently discovered that short-term administration of rapamycin reversed the aged oral cavity in mice by regenerating periodontal bone, alleviating gingival and periodontal bone inflammation and inducing revertive change of the oral microbiome toward to a more youthful composition (An et al., 2020). *In vitro*, aged human gingival fibroblasts (hGFs) treated with rapamycin (20 nmol/L) for 30 days to inhibit mTOR showed lower levels of senescence-associated markers ($p16^{INK4a}$, $p21^{CIP1a}$, IL-6, IL-8), reactive oxygen species (ROS) (catalase [Cat], superoxide dismutase [SOD2], peroxiredoxin 3 [Prdx3]), and inflammatory cytokines (IL-6 and IL-8) compared to the control (Xia et al., 2017).

3.4.2 Hepatic aging

The liver is the main base of the entire digestive system. Mitochondrial function and nutrient sensing pathway dysregulation are considered the most important keys to liver aging (Hunt et al., 2019). Aging liver can have serious negative impacts on digestive function and fitness. Rapamycin mitigated age-related liver degeneration (Wilkinson et al., 2012). Based on the unknown beneficial possibilities of rapamycin in attenuating old liver injury by activating autophagy and reducing oxidative stress through mTOR signaling, which are central in targeting the mechanism of aging, Martínez-Cisuelo et al. (2016) reported that rapamycin (14 mg/kg diet for 7 weeks continuously) treatment in natural middle-aged mice (16 months old) eliminated severe age-induced damage of mitochondrial ROS production, mitochondrial DNA (mtDNA) fragment accumulation inside nuclear DNA, mitochondrial protein lipoxidation, and lipofuscin accumulation, which are all related and which increase with age, as compared to young mouse livers (4 months old), by decreasing the amount of RAPTOR (an mTOR complex component) and increasing PGC1α and ATG13 protein levels (Martínez-Cisuelo et al., 2016). To identify the anti-aging effect of rapamycin in-depth, Fok et al. (2014) analyzed the liver transcriptome from male and female mice (25 months old) with 21-month dietary rapamycin treatment. Pathway analysis showed that mitochondrial function was the most significantly changed pathway among a total 13 significantly altered pathways in the rapamycin-fed female and male groups (Fok et al., 2014). Epigenetically, rapamycin impeded the increase and alteration in methylome disorder during aging in mouse liver (Wang et al., 2017). Devastating and imbalanced protein homeostasis is correlated to aging and age-related diseases (Ryazanov and Nefsky, 2002), while proteostasis failure is frequently linked with autophagy (Stead et

al., 2019). Therefore, from the protein viewpoint, the evaluation of liver proteome homeostasis using mRNA translation and protein turnover and abundance showed that 10-week rapamycin treatment increased 15% of protein half-lives and attenuated protein oxidative damage in 25-month-old mice compared to the age-matched controls. It is worth mentioning that mitochondrial dysfunction, i.e., the top pathway, was significantly altered by the 10-week rapamycin intervention, which agrees with the results of Fok et al. (Karunadharma et al., 2015).

3.4.3 Intestinal aging

Recently, the gut has become the hottest research target in the field of aging, which is attributed to its affinity with longevity because of the function of immunity and nutrition intake; several studies have revealed the close connection between fecal microbiota and lifespan (Barcena et al., 2019; Ding et al., 2019; Ruiz-Ruiz et al., 2019). Rapamycin mediated the fecal microbiome in aged mice, with a significant increase in the prevalence of segmented filamentous bacteria (Bitto et al., 2016). During aging in *Drosophila*, rapamycin maintained gut equilibrium by impeding intestinal stem cell proliferation and microbial expansion by inhibiting the mTOR pathway and inducing autophagy (Schinaman et al., 2019).

To sum up, rapamycin may exert useful protection against diseases of the aged digestive system.

3.5 Circulatory system

3.5.1 Cardiac-related disease

The heart is the organ that pumps blood around the body; it is the pacemaker and battery of all organs. With aging, the heart acquires dysfunction, which results in cardiovascular disease, influencing overall fragility and mortality, with total organ dysfunction. Three-month administration of rapamycin in old female C57BL/6J mice (24 months old) exerted beneficial effects on cardiovascular function and prevented age-related hypertrophy, with significant improvement in the 8.5% ejection fraction (%EF) and reduced left ventricular mass compared with controls via its anti-inflammatory impact, significantly altering four cytokines (G-CSF, LIX, IL-17, IL-7) (Flynn et al., 2013). Similarly, echocardiography in aged mice estimated that rapamycin decreased heart dimensional measures such as diastolic left ventricular internal diameter and overall heart mass (Neff et al., 2013). Myocardial fibrosis is an important and common characteristic of cardiac aging and eventually leads to myocardial dysfunction, increased stiffness, decreased cardiac compliance, decreased myocardial systolic function, and increased incidence of arrhythmia. In male Sprague-Dawley rats, 4-week rapamycin treatment attenuated cardiac fibrosis, with lower collagen 1 expression in the left ventricular myocardium, by inhibiting mTOR and MBG-mediated profibrotic signaling (Haller et al., 2016). In middle-aged dogs, echocardiography showed that short-term rapamycin treatment improved both diastolic and systolic age-related measures of heart function (early maximal ventricular filling velocity/late filling velocity [E/A] ratio, fractional shortening, EF) (Urfer et al., 2017).

3.5.2 Vascular aging

Several studies have noted and discussed the benefit of rapamycin for age-related vascular dysfunction. mTOR signaling activation leads to impaired endothelial function and foam cell formation by stimulating monocyte-to-macrophage transformation in the initial process of atherosclerosis (Cai et al., 2018). The inhibition of inflammation may be one of the main mechanisms. Delivering rapamycin in a biomimetic drug delivery system reduced vascular inflammation (MCP-1 and IL-β1) in mice, alleviating atherosclerosis progression (Boada et al., 2020). In old male B6D2F1 mice (30.3 ± 0.2 months), 6–8-week dietary rapamycin improved glucose tolerance and carotid artery endothelium-dependent dilation (EDD) while reducing aortic pulse wave velocity (PWV) (arterial stiffness) and collagen content via AMPK, PTEN, and p27Kip activation, and with reduced oxidative stress, compared to that in young mice (3.8 ± 0.6 months). This implied that rapamycin reduces oxidative stress by activating AMPK signaling, a senescence-related pathway, and increasing the expression of cell cycle–associated proteins to protect against age-related vascular dysfunction (Lesniewski et al., 2017). Apart from artery disease, rapamycin also exerts effects on venous disease. There is increased incidence of venous thromboembolism (VTE) in the elderly. In aged mice (16 months old) with experimental deep vein thrombosis (DVT), 2-month rapamycin (1.5 mg/kg/d intragastrically) treatment resulted in a significant reduction in susceptibility to DVT as compared to the aged controls. Mechanistically, rapamycin blocked the ROS production that increased with aging and induced mTORC1 activation in megakaryocytes and platelets (Yang et al., 2016).

In a clinical trial involving older adults with coronary artery disease, 12-week daily oral low-dose rapamycin had some positive effects on alleviating SASP (senescence-associated secretory phenotype) production, with increased IL-6 and decreased IL-8, but with no improvement in frailty (Singh et al., 2016). A clinical study that used rapamycin for treating carotid atherosclerosis in kidney transplant recipients showed that the rapamycin-treated group had higher total and high-density lipoprotein cholesterol levels and decreased carotid intima–media thickness (cIMT) at 6 and 12 months (Silva et al., 2018).

Hence, rapamycin may confer protective effects on the endothelium by attenuating ROS or inflammation in aging.

3.6 Musculoskeletal system

3.6.1 Age-related osteoporosis and sarcous aging

Bone loss is one of the normal features as people move towards old age rather than the exception, with the leading cause being bone marrow-derived mesenchymal stem cells (BMMSCs) manifesting imbalanced differentiation and reduced proliferation. mTORC1 expression in osteoclasts is believed to have an essential role in regulating bone homeostasis and mediating osteoporosis (Hiraiwa et al., 2019). *In vitro*, rapamycin (1.5 mg/kg every other day for 2 months) increased osteogenic differentiation and proliferative capacity, and decreased the adipogenic differentiation capacity of aged BMMSCs by activating autophagy to influence ROS and p53 levels. *In vivo*, intraperitoneal administration of rapamycin in a mouse model of senile osteoporosis

restored bone loss in aged mice (16 months old) (Ma et al., 2018). Moreover, by targeting mTOR, rapamycin attenuated osteogenesis and angiogenesis to improve bone mass in mice with iron accumulation, an independent risk factor for osteoporosis. That study indicated that inhibiting mTOR *in vivo* with rapamycin and *in vitro* with small interfering RNA (siRNA) transfection restored both osteogenesis and angiogenesis (Wu et al., 2019). Investigating if rapamycin has the same effect on aged skeletal muscle, Tang et al. (2019) reported that mice that had been treated with 14 ppm rapamycin from 9 months to 30 months of age had reduced oxidative stress and GDF expression, with reverted age-related muscle fiber loss via the suppression of AKT–mTORC1 signaling (Tang et al., 2019).

3.6.2 Age-related frailty

Motion is strongly associated with bone and muscle. Not only can rapamycin prevent age-related bone and muscle fiber loss, it can also attenuate age-related motion capacity. Old low-capacity runner (LCR) rats (16–22 months old) were fed rapamycin (2.24 mg/kg bw daily for 6 months). After 2-month administration of rapamycin, grip strength meter and maximum running distance (MRD) measurements showed that the former was significantly improved by about 13% and 60% in female and male rats, respectively, compared to baseline levels (when the rats had begun rapamycin treatment), and that MRD was increased by about 66% and 46% in female and male mice, respectively. Interestingly, compared to male mice, female mice reaped more benefit from the rapamycin treatment (Xue et al., 2016). A similar finding revealed that long-term (4–16 months of age) enteric rapamycin increased female C57BL/6J mouse grip strength and body mass, and reduced sleep fragmentation in C57BL/6J mice of both sexes (Fischer et al., 2015).

However, renal transplant recipients had abnormal bone marker profiles after rapamycin treatment, suggesting increased bone turnover and loss (Campistol et al., 2005). Notwithstanding, rapamycin was not identified to cause bone loss in rats (Goodman et al., 2001).

Overall, whether rapamycin is efficacious in preventing motor system aging in humans requires more solid evidence.

3.7 Respiratory system

3.7.1 Age-related pulmonary disease

Cellular senescence is now deemed an important element of the impelling mechanism for chronic lung diseases, especially chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), with irreversible decreased lung function resulting in decreased lung elasticity and enlargement of the alveolar spaces (Barnes et al., 2019). In addition, these lung pathologies are related to the involvement of mTOR signaling activation (Kennedy and Pennypacker, 2016). It is now thought that, like senescent cells, the fibroblasts and myofibroblasts from IPF are strongly resistant to apoptosis, while autophagy in the primary lung fibroblasts from IPF is much lower than that in young and age-matched normal lung fibroblasts via mTOR pathway mediation. Treatment with rapamycin and PP242, which block the PI3K–AKT–mTOR

signaling pathway, increased autophagy levels in an obvious manner, inducing persistent apoptosis in aged fibroblasts (Romero et al., 2016). Aging is one of the most pivotal risk factors of COPD, with progressive lung parenchymal damage leading to airflow obstruction and emphysema. Houssaini et al. (2018) found that lung cell senescence was associated with mTOR signaling activation; in transgenic mice, excessive mTOR pathway activation in the lung vascular cells or alveolar epithelial cells caused lung cell senescence and led to similar COPD lung pathological characteristics. However, low-dose (10 nM) rapamycin *in vivo* reduced the proinflammatory senescence-associated secretory levels in cells derived from COPD patients and from controls by inhibiting the mTOR pathway (Houssaini et al., 2018). Therefore, rapamycin leads to high improvement in respiratory system aging.

3.8 Endocrine system

3.8.1 Obesity and diabetes

Pancreas function declines gradually with lifespan prolongation, leading to increased morbidity of diabetes mellitus, a metabolic disease involving hyperglycemia and typically and closely connected with aging. Long-term blood glucose increases damage to the blood vessels and microvessels, resulting in dysfunction of the heart, brain, kidney, peripheral nerves, eyes, and feet, consequently seriously affecting overall health. Obesity is a major risk factor of diabetes. In a rat obesity model, rapamycin (1 mg/kg bw, 3 times/week) was administered to old rats (3 and 24 months old), and reduced adiposity and spared lean mass, decreasing blood triglycerides, increasing the lean/fat ratio, and normalizing elevated serum leptin by inhibiting mTORC1 signaling (Scarpace et al., 2016). Similar results showed that weekly rapamycin reduced obesity in male mice on a high-fat diet (Leontieva et al., 2014). mTOR pathway overactivation is associated with diabetes; therefore, rapamycin should be effective for improving diabetes (Blagosklonny, 2019; Condon and Sabatini, 2019). Some studies have shown that the benefits exerted by rapamycin include decreased body weight and improved insulin sensitivity in type 2 diabetic mice (Das et al., 2014; Deepa et al., 2013). Paradoxically, and confusingly, chronic treatment (15 days) with rapamycin led to glucose intolerance, hyperinsulinemia, and hyperglycemia in rats (Deblon et al., 2012). Similarly, Lamming et al. (2012) believed that chronic administration of rapamycin substantially impaired glucose homeostasis and insulin action in C57BL/6 mice by disrupting mTORC2 signaling (Lamming et al., 2012). The same phenotype has been found in kidney transplant recipients (Johnston et al., 2008). This is because the mTORC1 signal phosphorylates p70 S6 kinase and its downstream molecular target, S6, while mTORC2 regulates the insulin signaling pathway that involves PKC and AKT. Inhibiting mTORC2 mainly leads to altered metabolism; therefore, many of the detrimental consequences of rapamycin, such as serum glucose level fluctuations, may result from inhibiting mTORC2 (Lamming et al., 2012). Notably, significant glucose tolerance was not reversed in low-mTOR expression mice (mTOR Δ/Δ) as compared to wild-type mice (Wu et al., 2013). These controversial conclusions have not been clarified. It may be possible to propose the hypothesis that different drug approaches using windows and drug durations would generate these conflicting findings.

Meanwhile, rapamycin attenuated diabetic cardiac attacks, diabetic nephropathy, diabetic encephalopathy, foot ulcers, and vascular function (Choi et al., 2019; Dai et al., 2019; Das et al., 2014; Reifsnyder et al., 2018). However, despite the significant advantage of rapamycin in alleviating some diabetic complications, transient hyperglycemia, as an adverse effect of rapamycin, has also been observed (Reifsnyder et al., 2014). Rapamycin may remain ambiguous in balancing the glucose homeostasis of diabetes. However, the other benefits cannot be ignored.

In human studies, the prevalence of obesity was significantly lower in rapamycin-treated patients after liver transplantation as compared to controls (Dunkelberg et al., 2003).

3.9 Integumentary system

3.9.1 Skin aging

Skin aging is reflected in photodamage and dermal atrophy, with underlying tissue degeneration and barrier dysfunction. mTORC1 and mTORC2 signaling are specific and critical in epidermal barrier formation, and mTOR activation has recently been described as a feature of aging tissues and cells (Ding et al., 2016; Ding et al., 2020; Nacarelli et al., 2015). *In vitro*, rapamycin prevented ultraviolet A (UVA)-induced aging in human skin fibroblasts, in which mTOR was completely activated (Leontieva and Blagosklonny, 2017). Regarding the underlying molecular mechanism, mTORC2–Akt–IKKα signaling was reported as the targeting pathway involved in skin aging provoked by physical age and UV irradiation, and was ameliorated by rapamycin *in vivo* (Choi et al., 2016a).

In humans, rapamycin improvement of skin status was observed in most participants; immunohistochemical analysis revealed improvement in the histological appearance of skin tissue with the decreased expression of p16INK4A protein and increased collagen VII as compared to the placebo group (Chung et al., 2019).

3.9.2 Alopecia

Hair plays a pivotal role in conserving body heat, maintaining self-image, and preserving mental health. Hair loss, or alopecia, affects millions worldwide. It can occur due to aging, and methods for regrowing hair are lacking. Molecules that can promote hair follicle stem cell activation have been studied intensively, and the activation of autophagy by mTOR-inhibiting topical rapamycin treatment was deemed critical for stimulating hair regeneration *in vivo* in a C57BL/6J mouse model (Chai et al., 2019).

3.10. Immune system

Age-associated immune deficiency and remodeling of the immune system have been observed in the elderly. The immune system protects the body from changes that are not programmed in the body, and prevents the invasion of external substances. Therefore, the immune system regulates lifespan extension, and is associated with aging (Csaba, 2019). Clinically, rapamycin is used as an immunosuppressant for preventing

rejection in transplant patients, and there have been queries and limitations on the feasibility of rapamycin for delaying aging in humans due to its potential negative effect on immunity. However, rapamycin is now recognized as an optimal immunomodulator rather than an immunosuppressant (Araki et al., 2009). Other than improving immunogenicity in mice, long-term rapamycin also promoted innate lymphoid cell populations and functions in old C57 mice by inhibiting the mTOR pathway and enhancing the level of autophagy (Hurez et al., 2015; Jagannath et al., 2009). Although there has been no relevant clinical evaluation of the effect of rapamycin on immunity, the enhanced ability of vaccine response in the elderly by RAD001, a rapamycin analog, has been demonstrated (Mannick et al., 2014; Mannick et al., 2018).

In summary, rapamycin shows great potential for delaying aging in multiple organs comprehensively to achieve healthy aging. Although most of these studies were conducted on animal models, the potential capacity in humans is nevertheless shown (**Fig. 2**).

4. Toxicity and safety of rapamycin

Toxicity and safety are major concerns in pharmacotherapy. mTOR is extensively involved in numerous essential physiological processes, such as protein, nucleotide, and lipid synthesis. As an mTOR inhibitor, it is unsurprising that the use of rapamycin causes adverse reactions. Its safety and toxicity are summarized in the following two aspects: scientific and clinical study.

4.1 Scientific data

Few adverse effects of rapamycin in animals have been reported. A random controlled trial that evaluated the effects of short-term rapamycin treatment (10 weeks) in middle-aged dogs showed no clinical adverse effects in the rapamycin-treated group as compared to the placebo group. Hematological values remained within the normal range for all parameters studied; however, mean corpuscular volume (MCV) was decreased in the rapamycin-treated dogs (Urfer et al., 2017).

The adverse effects of topical rapamycin have not been reported in rapamycin-treated dog eyes, suggesting that 0.02% topical rapamycin might be an alternative treatment for canine patients with refractory dry eye (Spatola et al., 2018). In summary, animal studies have shown that rapamycin is relatively safe, with no significant adverse effects such as increased mortality or impaired organ function.

4.2 Clinical data

The immunosuppressive effect of rapamycin has led to it being used mainly in organ transplantation. The adverse events commonly linked to rapamycin in organ transplantation include hyperglycemia, lipid disorders, anemia, thrombocytopenia, arthralgia, acute renal toxicity, and delayed wound healing (Groth et al., 1999; Guilbeau, 2002). However, compared to other immunosuppressants such as glucocorticoids or

calcineurin inhibitors, the toxicities of long-term use of mTOR inhibitors in transplant patients are generally mild (Somers and Paul, 2015).

In the treatment of tuberous sclerosis complex (TSC), in which the underlying molecular mechanism is abnormal mTOR activation, the rapamycin-related common adverse events were stomatitis, upper respiratory tract infections, and nasopharyngitis (Li et al., 2019). Besides, of 25 patients enrolled in a 24-month nonrandomized, open-label trial estimating the efficacy of rapamycin in treating TSC or sporadic lymphangioleiomyomatosis, five patients had six serious adverse events, including diarrhea, pyelonephritis, stomatitis, and respiratory infections (Bissler et al., 2008). A clinical study reported consistent adverse effects (McCormack et al., 2011). Another study on TSC reported the following major adverse effects: oral aphthous ulcers (5/17), hypertriglyceridemia (3/17), microcytosis and hypochromia (3/17), diarrhea (2/17), acne (1/17), acute pyelonephritis (1/17), and proteinuria (1/17) (Cabrera López et al., 2011).

In the treatment of complicated vascular anomalies, a phase II clinical trial that enrolled a total of 61 patients found that in the 12-course rapamycin treatment (each course was defined as 28 days), grade ≥3 toxicities, i.e., blood/bone marrow toxicity, gastrointestinal toxicity, and metabolic/laboratory toxicity, were observed in 27%, 3%, and 3% of patients, respectively. No toxicity-related deaths occurred (Adams et al., 2016). Interestingly, a randomized control trial suggested that rapamycin effected no changes in cognitive and physical performance or self-perceived health status measure in elderly people during the study period. The adverse effects in the rapamycin group were facial rash (one participant), stomatitis (one event), and gastrointestinal events (two events), whereas placebo-treated participants only reported stomatitis (one event). Laboratory examination abnormalities were reflected in several erythrocyte indexes, including hemoglobin and hematocrit, red blood cell count (RBC), red blood cell distribution width (RDW), MCV, and mean corpuscular hemoglobin (MCH) (Kraig et al., 2018). Low-dose oral rapamycin increased the risk of menstrual cycle disturbances and ovarian cysts (Braun et al., 2012). A human study that estimated the efficacy and adverse effects of rapamycin on refractory/relapsed acquired pure red cell aplasia indicated tolerable adverse effects that included infections; mild oral mucositis; sinus tachycardia; elevated creatinine, transaminase, triglyceride, or cholesterol; and thrombocytopenia (Long et al., 2018).

In patients with active rheumatoid arthritis, low-dose rapamycin (0.5 mg on alternate days for 24 weeks) showed no evaluable adverse effects for immunoregulation therapy (Wen et al., 2019). In patients with Birt-Hogg-Dubé syndrome, a double-blind, random, facial left–right controlled trial of 0.1% topical rapamycin (6 months) showed that adverse effects were relatively more common but not significant after rapamycin treatment (68% of patients) than after placebo treatment (58% of patients; $p = 0.625$); the most frequent adverse effects were burning sensation, erythema, itching, and dryness (Gijezen et al., 2014). The results from a single cardiac transplant center showed that more than 70% of patients experienced an adverse effect caused by rapamycin, including infection, increased blood pressure and lipids, diarrhea, rash, mouth ulcers, pneumonitis, headache, arthralgias, worsening renal function, and

thrombotic thrombocytopenic purpura. The authors claimed that rapamycin commonly causes adverse effects and that drug discontinuance is often needed as appropriate, and that the adverse effects might be mainly connected with higher rapamycin blood concentration levels (9.1 ng/mL vs. 7.1 ng/mL, $p = 0.004$), suggesting that rapamycin tolerance may be enhanced if lower serum levels are maintained during treatment. Nevertheless, to improve tolerability, the potential for reducing pharmacodynamic considerations also needs to be balanced (Thibodeau et al., 2013). Menstrual cycle abnormalities and ovarian cysts also occurred during rapamycin treatment of patients with ADPKD (Braun et al., 2012). A review of the safety estimation of rapamycin in the treatment reported that the most frequent adverse effects were oral mucositis (31.9%), dyslipidemia (16.5%), leukopenia (12.3%), gastrointestinal symptoms (10.2%), and rash/eczema (8.2%) (Stallone et al., 2009).

In summary, the adverse effects of rapamycin include leukopenia, thrombocytopenia, hypertriglyceridemia, hypercholesterolemia, aphthous ulcers, edema, arthralgia, interstitial pneumonia, acne, delayed wound healing, sinus tachycardia, decreased renal function, gastrointestinal toxicity, rash, menstrual cycle disturbance, ovarian cyst, and infection. **Table 2** shows the commonly reported adverse events of rapamycin based on these systems, which correspond to the beneficial effect of rapamycin on organs. Several adverse effects have been observed, some of which even require complete drug withdrawal. An increasing amount of evidence continues to suggest that most of these adverse reactions are due to rapamycin-related dose dependence, and are reversible after ending treatment (Liu et al., 2014; Stallone et al., 2009). Moreover, to date, a total of 1399 clinical trials on rapamycin are listed on ClinicalTrials.gov (https://clinicaltrials.gov/), which indirectly illustrates the safety of rapamycin. Hence, the main advice for reducing the adverse effects of rapamycin are: (i) Precise screening of the treated population, and (ii) avoidance of persistent high doses (Stallone et al., 2009).

5. Discussion and future perspectives

Aging is a comprehensive, complicated, and progressive biological process with organ function decline that causes damaging physical changes. mTOR plays an essential role in the aging-related pathway network (Campisi et al., 2019). Rapamycin shows great potency for extending healthspan by blocking the mTOR pathway, which mediates a broad spectrum of biological activities such as cell growth, differentiation, apoptosis, metabolism, autophagy, inflammation, and mRNA translation (Barja, 2019; Johnson et al., 2015). Here, we describe the beneficial effects of rapamycin on aging organs and the age-related diseases of each system. Moreover, we summarize and discuss its toxicity and safety comprehensively. Further, there should be focus on the relevant adverse effects, which depend on the dose, administration route, duration, and health condition (Johnson and Kaeberlein, 2016). Intermittent dosing readily neutralizes the many adverse effects of rapamycin (Arriola Apelo et al., 2016). Hence, there is a need to explore the best means of administering rapamycin and balancing the trade-offs.

Much research has reported significant increases in lifespan influenced by rapamycin. Interestingly, several studies have indicated a sex difference in the effect of rapamycin on lifespan extension, being particularly notable in females (Harrison et al., 2009; Miller et al., 2011). Dosage may be one factor: Another study showed that, after receiving the low dose (feed; 126 ppm) at 20 months of age, both male and female mice had 50% longer lifespans than the blank group, and developed great progress in muscle strength and motor coordination. When given the higher dose (injection; 8 mg/kg/day), the male mice showed significantly increased life expectancy, but the female mice did not (Bitto et al., 2016). Therefore, more research is needed to verify the sex difference effects and to explore the underlying mechanisms.

To date, the clinical application of rapamycin remains limited by adverse events in both animals and humans. In diverse model organisms, the major molecular mechanism involved in decelerating aging and health promotion by rapamycin occurs primarily through mTORC1 signaling pathway inhibition (Morozumi and Shiozaki, 2021). However, mTORC2 is interrupted synchronously, and many adverse effects, both metabolic and immunological, occur as a result of the off-target effect during chronic exposure. Therefore, the various adverse effects of rapamycin and its analogs may be averted by inhibiting mTORC1 signaling selectively (Lamming et al., 2013). Schreiber et al. identified a novel rapamycin analog that is highly selective for mTORC1 to eliminate the adverse effects associated with rapamycin and its conventional analogs in mice (Schreiber et al., 2019). However, regarding both rapamycin and its analogs, additional validated studies are needed to provide applicable evidence for selecting an appropriate treatment and for prompting translation to human use with minimal adverse effects and maximum benefits.

The original occurrence and development of a disease is single-organ, but the aftershocks may be multi-organ. Pharmacological strategies are the major method for delaying aging and confronting age-related diseases. Based on the available data, rapamycin is a candidate with the most potential for achieving healthy aging. Nevertheless, contradictions and inconsistencies exist between human clinical studies and animal model studies. More prudent and data-rich studies are needed to identify the beneficial effects of rapamycin in delaying the aging pathologic processes and confronting the aging-related diseases.

Declaration of Competing Interest

None.

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Author Contributions

Conceptualization: YZ, JJZ; Writing - Original Draft: YZ; Writing - Review &

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Fig. 1. Effect of rapamycin on maximum lifespan extension in *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus* as a mammal example. The maximum lifespan extension was 19%, 14.5%, and 26% for *C. elegans* (Robida-Stubbs et al., 2012), *D. melanogaster* (Wang et al., 2016), and *M. musculus* (Miller et al., 2014), respectively. Abbreviations: AL, average lifespan; MLE, median lifespan extension.

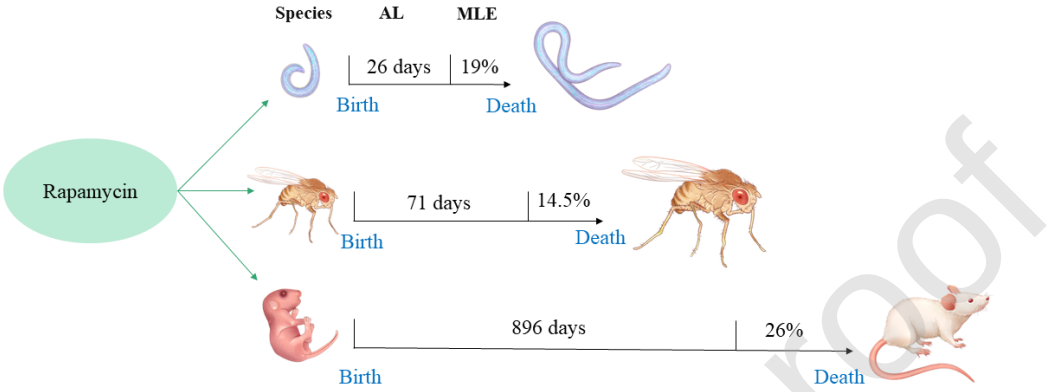
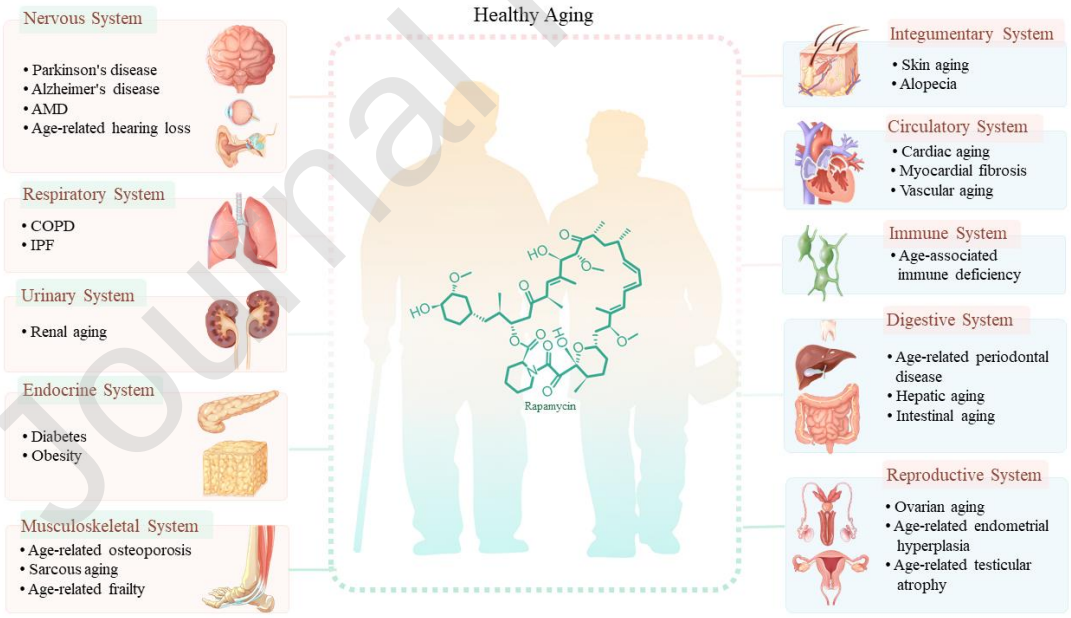


Fig. 2. The overall view of rapamycin in promoting healthy aging by attenuating aging in multiple organs.



Tables

Table 1 The role of rapamycin in anti-organ aging according to each system.

System	Organ aging/ age-related disease	Vivo/Vitro	Mode of action	Reference(s)
Nervous	Alzheimer disease	Rat	Activation of autophagy; PI3K–Akt1–mTOR–CREB signaling	(Singh et al., 2017b)
	Parkinson disease	Mouse	Activation of autophagy; PGC1α–TFEB signaling; anti-inflammation; inhibition of mTOR–Akt–NF-κB signaling	(Siddiqui et al., 2015b; Zhang et al., 2017)
	AMD	Rat	Activation of autophagy; inhibition of mTOR signaling	(Kolosova et al., 2012)
		RPE cell	Activation of autophagy; anti-inflammation; inhibition of mTOR	(Zhang et al., 2015b)
	ARHL	Mouse	Inhibition of mTORC1 signaling	(Fu et al., 2018)
		Mouse	Not described	(Altschuler et al., 2018)
Reproductive	Ovarian aging	Mouse	Inhibition of mTOR–FOXO3A and mTORC1 signaling	(Dou et al., 2017b; Garcia et al., 2019)
	POF	Mouse	Inhibition of PI3K–Akt–mTOR signaling	(Zhou et al., 2017)
	Endometrial hyperplasia	Mouse	Inhibition of mTOR signaling	(Bajwa et al., 2017)
	Testicular atrophy	Mouse	Not described	(Wilkinson et al., 2012)
Urinary	Renal aging	Mouse	Inhibition of mTOR signaling	(McNicholas et al., 2016)

Digestive	Periodontal disease	Mouse	Inhibition of mTOR signaling; anti-inflammation	(An et al., 2020; An et al., 2017)
		hGF	Inhibition of mTOR signaling; anti-inflammation and ROS	(Xia et al., 2017)
	Hepatic aging	Mouse	Inhibition of mTOR signaling; reducing ROS level and DNA damage; mitochondrial function	(Fok et al., 2014; Karunadharma et al., 2015; Martínez-Cisuelo et al., 2016)
	Intestinal aging	Mouse	Not described	(Bitto et al., 2016)
		Drosophila	Inhibiting mTOR signaling; inducing autophagy	(Schinaman et al., 2019)
Circulatory	Cardiac aging	Mouse	Anti-inflammation	(Flynn et al., 2013; Neff et al., 2013)
		Dog	Not described	(Urfer et al., 2017)
	Myocardial fibrosis	Rat	Inhibiting mTOR signaling; inhibiting Na–K–ATPase–MBG signaling	(Haller et al., 2016)
	Carotid artery dysfunction	Mouse	Reduction of oxidative stress; activation of AMPK and PTEN signaling; inhibiting mTOR signaling	(Lesniewski et al., 2017)
	Atherosclerosis	Mouse	Anti-inflammation	(Boada et al., 2020)
	Carotid atherosclerosis	Human	Not described	(Silva et al., 2018)
	Coronary artery disease	Human	Anti-inflammation	(Singh et al., 2016)

	VTE	Mouse	Inhibiting of mTORC1 signaling	(Yang et al., 2016)
Motor	Osteoporosis	Mouse	Inhibiting mTOR signaling; activating autophagy; reducing ROS level	(Ma et al., 2018; Wu et al., 2019)
	Aged skeletal muscle	Mouse	Reduction of oxidative stress inhibiting AKT–mTORC1 signaling	(Tang et al., 2019)
	Low motion capacity	Mouse	Not described	(Fischer et al., 2015; Xue et al., 2016)
Respiratory	IPF	Cell	Inhibiting PI3K–AKT–mTOR signaling; activation of autophagy	(Romero et al., 2016)
	COPD	Cell	Inhibiting mTOR signaling	(Houssaini et al., 2018)
Endocrine	Obesity	Rat	Inhibiting mTORC1 signaling	(Leontieva et al., 2014; Scarpace et al., 2016)
	Diabetes	Mouse	Inhibiting mTOR signaling; attenuating oxidative stress	(Blagosklonny, 2019; Condon and Sabatini, 2019; Das et al., 2014; Deepa et al., 2013)
Integumentary	Skin aging	Mouse	Inhibiting mTORC2–Akt–IKKα signaling	(Choi et al., 2016b)
		Human	Not described	(Chung et al., 2019)
	Alopecia	Mouse	Inhibiting mTOR signaling	(Chai et al., 2019)
Immune	Age-associated immune deficiency	Mouse	Inhibiting mTOR signaling; improvement of autophagy	(Hurez et al., 2015; Jagannath et al., 2009)

Table 2 The clinical and scientific systematic adverse effects of rapamycin.

System	Adverse effect	Species	Reference(s)
Nervous	Headache	Human	(Thibodeau et al., 2013)
Reproductive	Menstrual cycle disturbance	Human	(Braun et al., 2012)
	Ovarian cyst	Human	(Braun et al., 2012)
Urinary	Acute renal toxicity	Human	(Groth et al., 1999)
	Worsening renal function	Human	(Thibodeau et al., 2013)
	Acute pyelonephritis	Human	(Cabrera López et al., 2011)
	Proteinuria	Human	(Cabrera López et al., 2011)
	Elevated creatinine	Human	(Long et al., 2018)
Digestive	Oral aphthous ulcers	Human	(Cabrera López et al., 2011; Thibodeau et al., 2013)
	Stomatitis	Human	(Kraig et al., 2018; Long et al., 2018)
	Diarrhea	Human	(Cabrera López et al., 2011; Thibodeau et al., 2013)
	Acne	Human	(Cabrera López et al., 2011)
	Gastrointestinal toxicity	Human	(Adams et al., 2016; Kraig et al., 2018)
	Transaminase	Human	(Long et al., 2018)
	Thrombocytopenia	Human	(Long et al., 2018)
Circulatory	Anemia	Human	(Groth et al., 1999)
	Increase in blood pressure	Human	(Thibodeau et al., 2013)
	Thrombocytopenia	Human	(Groth et al., 1999; Thibodeau et al., 2013)
	Blood/bone marrow toxicity	Human	(Adams et al., 2016)
	Erythrocyte abnormality	Human/Dog	(Kraig et al., 2018; Urfer et al., 2017)
	Sinus tachycardia	Human	(Long et al., 2018)
Motor	Arthralgia	Human	(Groth et al., 1999; Thibodeau et al., 2013)

	Microcytosis and hypochromia	Human	(Cabrera López et al., 2011)
Respiratory	Pneumonitis	Human	(Thibodeau et al., 2013)
Endocrine	Hyperglycemia	Human	(Groth et al., 1999)
	Lipid disorders	Human	(Cabrera López et al., 2011; Groth et al., 1999; Long et al., 2018; Thibodeau et al., 2013)
Integumentary	Delayed wound healing	Human	(Guilbeau, 2002)
	Facial rash	Human	(Gijezen et al., 2014; Kraig et al., 2018; Thibodeau et al., 2013)
	Burning sensation	Human	(Gijezen et al., 2014)
	Itching	Human	(Gijezen et al., 2014)
	Dryness	Human	(Gijezen et al., 2014)