Targeting ageing with rapamycin and its derivatives in humans: a systematic review

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Rapamycin and its derivatives (rapalogs) are inhibitors of mTOR, a major regulator of the ageing process. We aimed to summarise the effects of rapamycin and its derivatives on the severity of ageing-related physiological changes and disease in adults. A search across five databases yielded 18 400 unique articles, resulting in 19 included studies. Rapamycin and its derivatives improved physiological parameters associated with ageing in the immune, cardiovascular, and integumentary systems of healthy individuals or individuals with ageing-related diseases. Overall, no significant effects on the endocrine, muscular, or neurological systems were found. The effects of rapamycin or its derivatives on the respiratory, digestive, renal, and reproductive systems were not assessed. No serious adverse events attributed to rapamycin and its derivatives were reported in healthy individuals; however, there were increased numbers of infections and increases in total cholesterol, LDL cholesterol, and triglycerides in individuals with ageing-related diseases. Future studies should assess the remaining unexamined systems and test the effects of long-term exposure to rapamycin and its derivatives.

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See **Online** for appendix

Introduction

Although life expectancy is increasing, healthy life expectancy is not increasing at the same pace, leading to more years being lived in poor health.[1] Therefore, the disease burden is shifting to chronic disease, with age being the main driver for chronic ageing-related diseases.[2] The average amount of time spent living with chronic disease in industrialised countries is about 11 years.[3] As a result, there is a growing focus on research that targets ageing,[4] for example the investigation of geroprotectors, compounds capable of slowing the ageing process[5] and delaying the onset of multiple tissue dysfunction and ageing-related diseases.

At least five of 12 defined hallmarks of ageing are modulated by the mechanistic target of rapamycin (mTOR) pathway.[5–7] The mTOR pathway has been linked to multiple chronic disease processes, such as declining immune function,[8] deteriorating pulmonary function (leading to chronic obstructive pulmonary disease),[9] diminishing bone mineral density (leading to osteoporosis),[10] development of cancer,[11,12] atherosclerosis and cardiac hypertrophy in cardiovascular disease,[13–15] and neurodegeneration.[16] Rapamycin and its derivatives are inhibitors of mTOR. These drugs are approved for use in anticancer therapies, rejection prophylaxis after organ transplant, drug-eluting coronary stents, and the treatment of lymphangioleiomyomatosis and tuberous sclerosis.[17]

Animal studies have shown that decreased mTOR signalling extends lifespan by up to 20% in yeast,[18,19] 19% in worms,[20,21] 24% in flies,[22,23] and 60% in mice.[23–25] In humans, randomised controlled trials have shown that the administration of rapamycin derivatives alongside vaccines against seasonal influenza can boost immune response by reversing immunosenescence.[26,27] We aimed to summarise the effects of rapamycin and its derivatives on the severity of ageing-related physiological changes and disease in adults.

Methods

Search strategy and selection criteria

The protocol of this systematic review was registered with PROSPERO (CRD42022345827). We searched PubMed, Embase, SCOPUS, Cochrane, and Web of Science for full-text articles published from database inception to July 18, 2022. Key search terms included "rapamycin", "sirolimus", "everolimus", and "temsirolimus" (appendix pp 1–14).

Articles included in this systematic review met the following criteria: the mean or median age of the study cohort was 18 years or older; outcomes on ageing-related physiological changes or disease were reported; and they were interventional studies with rapamycin or its derivatives (no restriction on the method). Articles were excluded if in vitro or animal models were used; rapamycin or its derivatives were given with the primary purpose of treating cancer or preventing rejection of transplants or implants; they were conference abstracts, clinical study protocols, reviews, editorials, letters to the editor, or book chapters; fewer than five participants were included in the study; or the articles were published in a language other than English.

After duplicates were removed, two reviewers (DJWL and AHK) independently screened the titles and abstracts and, subsequently, the full-text articles of potentially relevant studies against the inclusion and exclusion criteria. A third reviewer (ABM) resolved any disagreements. The articles were organised and managed using Covidence systematic review software (Veritas Health Innovation, Melbourne, VIC, Australia).

To establish the progress of rapamycin and its derivatives in ongoing human studies, we searched ClinicalTrials.gov for relevant trials from database inception to July 2, 2023. No new trials have been marked as completed and published after the last date of our search on July 2, 2023.

Data extraction and assessment

The following variables were extracted independently by two reviewers (DJWL and AHK): author, year of publication, study design, sample size (treatment group and control group), age, sex, ageing-related disease of the cohort, duration of the study, outcomes, dosage form (eg, capsule, tablet, or liquid), dose, dosing interval, intervention duration, route of administration, comparator, setting, and primary and secondary outcomes. For binary outcomes, DJWL and AHK extracted numbers of events, percentages of events, or ratios with CIs. For continuous outcomes, DJWL and AHK extracted mean or median value, standard deviation, standard error, CI or IQR, mean difference, and p values. Outcomes were categorised according to the physiological systems identified by the American Physiological Society.[28] The overall efficacy of rapamycin and its derivatives on physiological systems was evaluated using the following categories: positive, partially positive, no effect, negative, and partially negative (the criteria for these categories are listed in the appendix [p 15]).

For more on the **Cochrane risk-of-bias tool** see https://methods.cochrane.org/risk-bias-2

Study bias was assessed by two independent reviewers (DJWL and AHK) using the Cochrane risk-of-bias tool (version 2.0). Conflicts were resolved by a third reviewer (ABM). Five key sources of bias were assessed: the randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported results. The key sources of bias were denoted as high risk or low risk, depending on whether the aspect was deemed to encourage or mitigate bias, or as some concerns, if the aspect was not reported. The overall risk of bias in a study was classified as low if all key domains were low risk, high if one or more of the domains were denoted as high risk, and some concerns if one or more of the domains were denoted as some concerns.

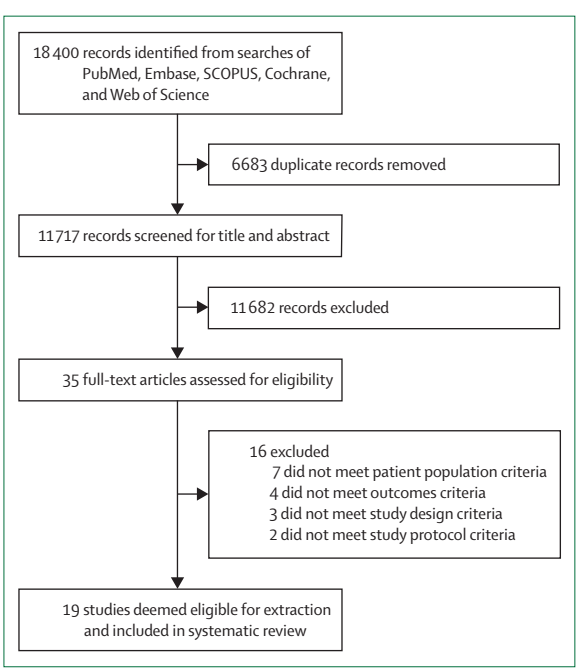

Figure 1: **Flowchart for study selection**

Results

We found 18 400 articles; after excluding 6683 duplicates, the titles and abstracts of 11 717 articles were screened. 35 articles underwent full-text screening, and 19 articles were included in this Review (figure 1).

Ten articles included healthy individuals,[26,27,29–36] with two of these articles reporting on two[36] and three[27] studies each, resulting in a total of 13 studies in healthy individuals. These 13 studies included 2077 individuals (981 [47·2%] men, median or mean age 25·5 years[33] to 81 years in the intervention group[36]), with cohort sizes ranging from six[31] to 1021 (table).[27]

Nine articles reported data on individuals with ageing-related diseases. Of these nine studies, six focused on neurodegenerative disorders,[37–42] one on pulmonary disorders,[43] and two on rheumatological disorders.[44,45] 401 individuals (mean or median age 50·3 years[45] to 84·5 years[39] in the intervention groups) were included in these studies, and cohort size ranged from six[42] to 121 (table).[44]

In healthy individuals, rapamycin was the most studied drug (seven studies),[30–33,35,36] followed by RTB101 (three studies),[27] everolimus (two studies),[26,34] and temsirolimus (one study).[29] In individuals with ageing-related diseases, rapamycin was the most tested drug (seven studies),[37–42,45] followed by everolimus (two studies).[43,44] Rapamycin and rapamycin derivatives had varying effects on physiological systems (figure 2, appendix pp 16–63), with varying dose–effect relationships (figure 3).

Rapamycin (1 mg/day orally, for 8 weeks) did not affect the cognitive function of healthy individuals, assessed using the Executive Interview 25, St Louis University Mental Status Exam, and Texas Assessment of Processing Speed.[36] In addition, in patients with multiple system atrophy treated with rapamycin (2–6 mg/day orally, for 48 weeks), there were no significant changes in Unified Multiple System Atrophy Rating Scale total scores or differences in right or left putaminal diffusivity and volume; there were also no changes in other parameters or brain regions.[41] The same study showed that following treatment with rapamycin, average retinal nerve fibre layer thickness and macular ganglion cell complex thickness were unchanged, and these parameters were also unchanged in specific quadrants of both eyes; the plasma concentrations of neurofilament light chain and α-synuclein-containing exosomes were also unchanged after rapamycin treatment.[41] In individuals with persistent wet, age-related macular degeneration who were given 440 µg intravitreal rapamycin every 2 months, (three doses),[39] or individuals with geographic atrophy in age-related macular degeneration who were given 440 µg every 2 months, for 24 months,[42] central subfield

	Study location	Study design	Intervention	Comparator	Study duration, days	Cohort size, N	Age, years*	Study participants by sex, N	Health status
Studies on healthy individuals									
Boni et al (2012)[29]	USA	Crossover	Temsirolimus	Placebo + moxifloxacin	2	58	18·0–50·0	0 women, 58 men	Healthy
Chung et al (2019)[30]	USA	RCT	Rapamycin	Placebo	240	36	>40·0	28 women, 8 men	No major morbidities but evidence of age-related photoageing and dermal volume loss
Dickinson et al (2013)[31]	USA	Crossover	Rapamycin	Self	2	6	26·0	3 women, 3 men	Healthy
Drummond et al (2009)[32]	USA	RCT	Rapamycin	No intervention	1	Overall 15, intervention 7, control 8	29·0	0 women, 15 men	Healthy
Gundermann et al (2014)[33]	USA	RCT	Rapamycin + blood flow restriction	Blood flow restriction exercise	2	16	25·5	0 women, 16 men	Healthy
Hörbelt et al (2020)[34]	Germany	Pilot study	Everolimus	Self	15	Overall 19, intervention one 6, intervention two 7, intervention three 6	Overall 28·2, intervention one 27·8, intervention two 27·7, intervention three 28·8	Intervention one: 0 women, 6 men; intervention two: 0 women, 7 men; intervention three: 0 women, 6 men	Healthy
Krebs et al (2007)[35]	Austria	Crossover study	Rapamycin	Placebo	2	Overall 11, intervention one 8, intervention two 3	Overall 28·3, intervention one 29·0, intervention two 26·0	Intervention one: 0 women, 8 men; intervention two: 0 women, 3 men	Healthy, without a family history of diabetes, dyslipidaemia, or conditions related to insulin resistance, and not taking any medication
Kraig et al (2018)[36]	USA	RCT phase 1	Rapamycin	Placebo	120	Overall 8, intervention 4, control 4	81·0–95·0	Intervention: 0 women, 4 men; control: 0 women, 4 men	All chronic diseases (eg, hypertension and coronary artery disease) clinically stable
Kraig et al (2018)[36]	USA	RCT phase 2	Rapamycin	Placebo	60	Overall 17, intervention 7, control 10	70·0–95·0; intervention group 74·8†	Intervention: 2 women, 5 men; control: 5 women, 5 men	All chronic diseases (eg, hypertension and coronary artery disease) clinically stable
Mannick et al (2021)[27]	New Zealand	RCT phase 2b (part 1)	RTB101	Placebo	..	Overall 179, intervention one 61, intervention two 58, control 60	Overall 74·9, intervention one 74·0, intervention two 76·5, control 74·4	Intervention one: 28 women, 33 men; intervention two: 27 women, 31 men; control: 24 women, 36 men	With asthma, type 2 diabetes, chronic obstructive pulmonary disease, or congestive heart failure
Mannick et al (2021)[27]	USA	RCT phase 2b (part 2)	RTB101	Control one, RTB101 + everolimus; control two, placebo	168	Overall 473, intervention one 118, intervention two 120, control one 115, control two 120	Overall 73·3, intervention one 73·1, intervention two 73·0, control one 73·9, control two 73·2	Intervention one: 66 women, 52 men; intervention two: 59 women; 61 men; control one: 58 women, 57 men; control two: 67 women, 53 men	With asthma, type 2 diabetes, chronic obstructive pulmonary disease, or congestive heart failure
Mannick et al (2021)[27]	New Zealand and Australia	RCT phase 3	RTB101	Placebo	80	Overall 1021, intervention 511, control 510	Overall 72·8, intervention 72·6, control 73·1	Intervention: 292 women, 219 men; control: 286 women, 224 men	Without chronic obstructive pulmonary disease or other significant pulmonary disease other than asthma, current evidence of unstable cardiac condition or other serious or unstable medical disorder.
Mannick et al (2014)[26]	New Zealand and Australia	RCT	RAD001	Placebo	70	Overall 218, intervention one 53, intervention two 53, intervention three 53, control 59	Overall 71·3, intervention one 70·8, intervention two 72·0, intervention three 71·4, control 71·1	Intervention one: 19 women, 34 men; intervention two: 26 women, 27 men; intervention three: 21 women, 32 men; control: 28 women, 31 men	Without unstable medical conditions

(Table continues on next page)

	Study location	Study design	Intervention	Comparator	Study duration, days	Cohort size, N	Age, years	Study participants by sex, N	Health status
(Continued from previous page)									
Studies on individuals with age-related diseases									
Bruyn et al (2008)[44]	Europe and USA	RCT	Everolimus	Placebo	84	Overall 121, intervention 61, control 60	Overall 54·5†	Overall: 93 women, 28 men†	Rheumatoid arthritis
Dugel et al (2012)[37]	USA	RCT	Rapamycin (subconjunctival)	Rapamycin (intravitreal)	365	Overall 50, subconjunctival 25, intravitreal 25	Overall 63·5, subconjunctival 64·0, intravitreal 63·0	Subconjunctival: 13 women, 12 men; intravitreal: 14 women, 11 men	Diabetic macular oedema
Gensler et al (2018)[38]	USA	RCT	Rapamycin	Lidocaine	365–730	Overall 52, intervention 27, control 25	Overall 79·1, intervention 78·5, control 79·8	Intervention: 17 women, 10 men; control: 12 women, 13 men	Geographic atrophy in age-related macular degeneration
Minturn et al (2021)[39]	USA	RCT	Rapamycin	Bevacizumab or aflibercept	182·5	Overall 40, intervention 20, control 20	Overall 81·8, intervention 84·5, control 79·2	..	Persistent exudative age-related macular degeneration
Nussenblatt et al (2010)[40]	USA	RCT phase 1/2	Rapamycin	Observation	182·5	Overall 13, intervention (rapamycin) 3, intervention (daclizumab) 4, intervention (infliximab) 3, control (observation) 3	Overall 80·3, intervention (rapamycin) 87·0, intervention (daclizumab) 79·2, intervention (infliximab) 77·3, control (observation) 78·0	Intervention (rapamycin): 3 women, 0 men; intervention (daclizumab): 4 women, 0 men; Intervention (infliximab): 2 women, 1 man; control: 3 women, 0 men	Age-related macular degeneration with choroidal neovascularisation
Palma et al (2022)[41]	USA	RCT	Rapamycin	Placebo	406	Overall 47, intervention 35, control 12	Overall 58·5 intervention 59·0, control 58·0	Intervention: 14 women, 21 men; control: 6 women, 6 men	Parkinsonian-predominant or cerebellar predominant multiple system atrophy
Petrou et al (2014)[42]	USA	RCT	Rapamycin	Fellow eye, no treatment	730	6	74·3	2 women, 44 men	Geographic atrophy
Seyfarth et al (2013)[43]	Germany	RCT	Everolimus ‡ conventional treatment	None	180	10	51·6	6 women, 4 men	Pulmonary hypertension
Wen et al (2019)[45]	China	RCT	Rapamycin + conventional treatment	Methotrexate, leflunomide, hydroxychloroquine, or thalidomide	168	Overall 62, intervention 42, control 20	Intervention 50·3, control 51·8	Intervention: 34 women, 8 men; control: 17 women, 3 men	Rheumatoid arthritis

RCT=randomised controlled trial. *Ages are mean values for all studies unless otherwise indicated, except for the study of Palma and colleagues,[43] which shows median values. †Exact data unavailable for the overall and control groups. ‡Data were not provided separately for control and intervention groups.

Table: **Characteristics of studies examining the effects of rapamycin and its derivatives on healthy individuals and individuals with age-related diseases**

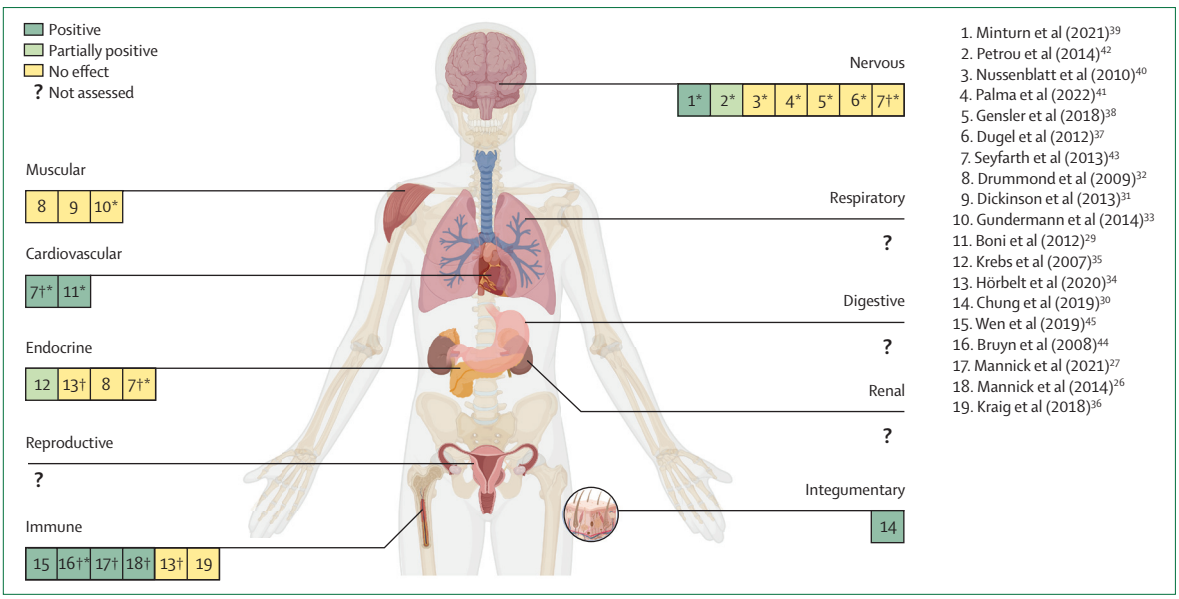

Figure 2: **Summary of the overall effect of rapamycin and its derivatives on physiological systems in healthy individuals and individuals with ageing-related diseases**
Figure created with BioRender.com. *Studies on individuals with age-related diseases. †Studies using rapamycin derivatives.

thickness was reduced at 6 months[39] and 12 months[42] compared with the control eye[42] or control treatment.[39] In these two studies,[39,42] choroidal neovascularisation was reduced and there was associated loss of visual acuity[42] when rapamycin was given for a longer duration. In addition, rapamycin had no significant effect on visual acuity in individuals with age-related macular degeneration[38] or diabetic macular oedema.[37]

In individuals with age-related macular degeneration, rapamycin (440 µg/month intravitreally, for 24 months) was not associated with any reduction in geographic atrophy area or change in halo progression, compared with the placebo group.[38] A decreased need for anti-VEGF was seen in individuals with age-related macular degeneration after treatment with rapamycin (2 mg orally, every other day, for 6 months), but there were no significant changes in visual acuity or recurrence of intraretinal or subretinal fluid, as evaluated by optical coherence tomography, in patients treated with rapamycin, compared with the observation group.[40] In addition, compared with placebo, rapamycin (440 µg intravitreally, every 2 months, for 24 months) had no significant effect on the geographic atrophy area or the mean number of scotomatous points in individuals with geographic atrophy.[42] Finally, intravitreal or sub-conjunctival rapamycin was associated with retinal thinning, compared with placebo[42] or baseline.[37]

In healthy individuals, treatment with temsirolimus (25 mg intravenously, once) led to a significant increase in heart rate 16–48 h after dosing, with no effect on corrected QT interval.[46] In patients with pulmonary hypertension, treatment with everolimus (0·75 mg orally, every 12 h, for 2 days, followed by adjustments to maintain target serum concentrations at 5–8 ng/mL over 6 months) resulted in significant enhancements in cardiac output relative to body surface area, compared with baseline measurements.[43] Moreover, this treatment led to reductions in both pulmonary vascular resistance and pulmonary arterial pressure. Several other

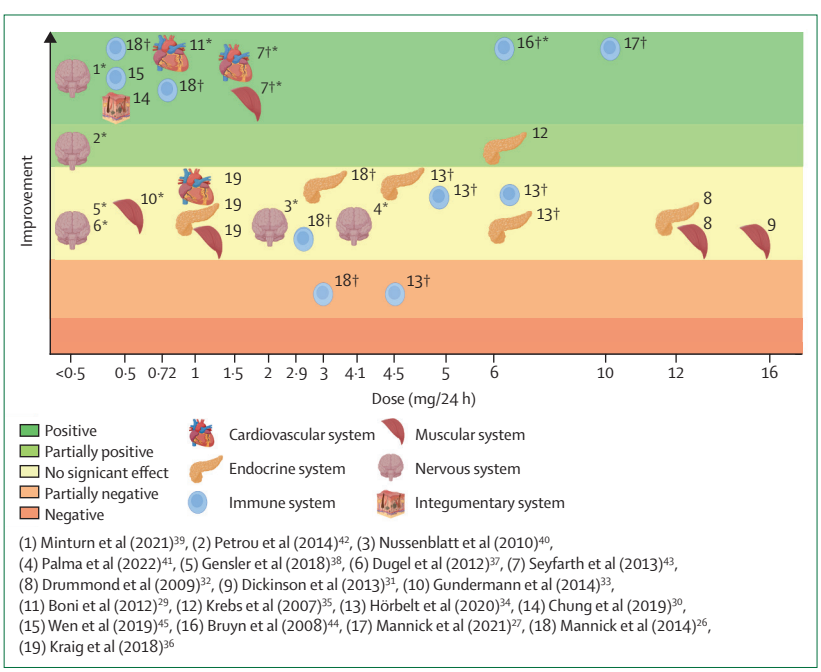

Figure 3: **Rapamycin and its derivatives; dose–effect relationship on physiological systems in healthy individuals and individuals with ageing-related diseases**
Figure created with BioRender.com. *Studies on individuals with age-related diseases. †Studies using rapamycin derivatives.

parameters also showed positive trends towards improvement, including right atrial pressure and pulmonary capillary wedge pressure (both decreased), maximum rate of oxygen consumption (increased), cardiac workload (reduced), and NT-pro-BNP concentration (decreased).[43]

Rapamycin (6 mg orally, once) was associated with a significant increase in glucose turnover in healthy individuals with induced peripheral hyperinsulinaemia.[35] At a lower dose, rapamycin (1 mg/day orally, for 8 weeks) did not have a significant effect on plasma concentrations of fasting glucose, random glucose, or glycated haemoglobin A_{1c}, or on insulin concentration or sensitivity (assessed using the homoeostatsis model assessment of insulin resistance and Matsuda index) following a glucose tolerance test in healthy individuals.[36]

Compared with placebo, rapamycin (12 mg orally, once, 2 h before exercise) had no effect on post-exercise blood insulin concentrations in healthy individuals.[32] In addition, compared with placebo, rapamycin (6 mg orally, once) did not affect post-amino acid infusion fasting plasma insulin concentrations or concentrations of C-peptide, glucose, or glucagon in healthy individuals.[35]

Everolimus (3 mg orally, every 12 h for 3 days; ie, four doses) reduced plasma and salivary cortisol concentrations in healthy individuals.[34] Rapamycin (12 mg orally, once, 2 h before exercise) had no effect on post-exercise blood cortisol concentrations.[32] In addition, compared with placebo, rapamycin (6 mg orally, once) given to healthy individuals following amino acid infusion had no effect on fasting plasma cortisol concentrations, plasma growth hormone concentrations, or fasting plasma free fatty acids concentrations.[35] Rapamycin (1 mg/day orally, for 8 weeks) has also been shown to have no effect on plasma lipids following a glucose tolerance test in healthy individuals.[36] Another study in healthy individuals showed that plasma noradrenaline concentrations were significantly increased in the everolimus group (1·5 mg, 2·25 mg, or 3 mg orally, every 12 h for 3 days; ie, four doses), compared with the placebo group.[34]

In patients with rheumatoid arthritis, everolimus (6 mg/day orally, for 12 weeks) was associated with modest increases in the concentrations of total cholesterol, LDL cholesterol, and triglycerides, which returned to baseline at the end of treatment.[44] In patients with pulmonary hypertension taking everolimus (0·75 mg orally, every 12 h for 2 days, followed by adjustments to maintain target serum concentrations at 5–8 ng/mL over 6 months), a significant increase in concentrations of total cholesterol and triglycerides, where observed, when compared to baseline.[43] Rapamycin (2 mg orally, every other day, for 6 months) had no effect on cholesterol concentrations in patients with choroidal neovascularisation.[40]

Three studies explored the effect of rapamycin on mixed muscle protein fractional synthetic rate (FSR) in healthy individuals.[31–33] In two of these studies, which used different rapamycin dosages (12 mg orally, once, 2 h before exercise;[32] or 16 mg orally, once, 1 h before exercise[33]), no significant changes in FSR were observed after low-intensity resistance exercise, compared with baseline. The third study showed that rapamycin (16 mg orally, once) did not affect mixed muscle protein FSR at rest.[31]

Other results from these studies showed that post-exercise blood concentrations of isoleucine, valine, and phenylalanine were not significantly different in rapamycin-treated (12 mg orally, once, 2 h before exercise) and placebo groups, whereas the leucine concentration was significantly higher 1 h post exercise in the rapamycin-treated group.[32] In addition, the phenylalanine concentration at rest did not differ in groups receiving rapamycin (16 mg orally, once) or placebo, and there was no difference in muscle fractional breakdown rate in individuals treated with rapamycin after exercise (16 mg orally, once, 1 h before exercise)[33] or at rest (16 mg orally, once).[31]

A study assessing physical function in healthy individuals aged 70–95 years found no significant change in grip strength or walking speed following treatment with rapamycin (1 mg/day orally, for 8 weeks).[36] In individuals with pulmonary hypertension,[43] there was a non-significant increase in 6-min walk distance after 6 months of everolimus treatment (0·75 mg orally, every 12 h for 2 days, then adjusted to target serum concentrations of 5–8 ng/mL for 6 months).

In healthy individuals, 10 μM rapamycin (0·5 mL topically, every 24–48 h for 8 months) significantly reduced the expression of p16INK4A, a marker of skin senescence, in the epidermal layer of the skin.[30] Other markers, such as p21Cip2 and TP53, showed trends towards reduction, but these changes did not reach significance. Among mRNA markers relevant to senescence and skin, only collagen type VII mRNA levels showed significant changes.[30] We did not find any studies that assessed the effects of rapamycin or its derivatives on ageing-related skin disease.

Seven studies (in four articles) assessed the effects of rapamycin or its derivatives on the immune system in healthy individuals,[26,27,34,36] of which four studies (in two articles) related to vaccination.[26,27] Treatment with everolimus (0·5 mg/day or 5 mg/week orally, for 6 weeks) significantly increased the response to seasonal influenza vaccine, particularly in those with low baseline influenza titres.[26] RTB101 (10 mg/day orally, for 16 weeks) significantly increased interferon-induced antiviral responses and reduced the incidence of laboratory-confirmed respiratory tract infections, especially in those aged 85 years or older; these effects were not seen with lower doses, more frequent dosing, or when RTB101 was combined with everolimus.[27]

The effects of rapamycin or its derivatives on T cells and B cells were inconsistent across studies. In one study, the numbers of PD-1-positive CD4 and CD8 T cells

were decreased in pooled everolimus groups (0·5 mg/day, 5 mg/week, and 20 mg/week, for 6 weeks) compared with the placebo group.[26] In another study, no significant changes in these cells were observed following rapamycin treatment (1 mg/day orally, for 8 weeks).[36]

Serum cytokines did not change following rapamycin treatment (1 mg/day orally, for 8 weeks), apart from an increase in TNF-α concentration.[36] Secretion of IL-10 by stimulated peripheral blood mononuclear cells was significantly reduced following everolimus treatment (1·5 mg, 2·25 mg, or 3 mg orally, every 12 h, over 3 days; ie, four doses)[34] but not following rapamycin treatment (1 mg/day orally, for 8 weeks).[36]

Everolimus (6 mg/day orally, for 12 weeks) plus methotrexate was associated with significant improvement in rheumatoid arthritis activity, compared with the control treatment, as evaluated using the American College of Rheumatology criteria.[44] Another study showed that the combined rheumatoid arthritis 28-joint disease activity score and erythrocyte sedimentation rate were not significantly different in groups receiving rapamycin (0·5 mg every other day, orally, for 24 weeks) plus conventional treatment or conventional treatment only.[45] In addition, in patients with rheumatoid arthritis, the number of tender joints was significantly reduced following treatment with everolimus plus conventional treatment (6 mg/day orally, for 12 weeks; compared with placebo plus conventional treatment)[44] or rapamycin (0·5 mg orally, every other day, for 6 months) plus conventional treatment (compared with conventional treatment alone).[45] Other markers of disease activity of rheumatoid arthritis reflected by numbers of swollen joints, patient pain scale, patient-assessed global disease activity, and physician-assessed global disease activity were significantly improved in the everolimus (6 mg/day orally, for 12 weeks) plus conventional treatment group, compared with the conventional treatment group.[44]

There were no serious adverse events that were related to rapamycin or its derivatives in any studies including healthy individuals. Serious adverse events were defined as those that, according to the investigator or sponsor, resulted in any of the following outcomes: death, a life-threatening adverse event, admission to hospital or prolongation of existing hospitalisation, persistent or substantial incapacity or disruption of the ability to conduct normal life functions, or a congenital anomaly or birth defect attributed directly to the intervention drug in any of the studies.[47] The adverse events in the included studies were generally classified as mild or moderate and were reversible after the discontinuation of treatment (appendix pp 67–82). For example, a study conducted among healthy individuals taking rapamycin (1 mg/day orally, for 8 weeks) reported a within-group increase in serum triglyceride concentration of about 40% above baseline (p=0·05); however, this change was not statistically significant when compared with the placebo group (p=0·12).[36] Additionally, a study on patients with multiple system atrophy reported a significantly higher number of adverse effects in the rapamycin group (2–6 mg/day orally, for 48 weeks) compared with the placebo group; the most prevalent adverse effects in this study were infections (urinary tract, upper respiratory, and skin infections and sinusitis) and oral and labial pathology (aphthae, gingivitis, and herpes-like vesicles).[41] A study on patients with pulmonary hypertension reported a statistically significant increase in concentrations of total cholesterol and triglycerides in the group taking everolimus (0·75 mg orally, every 12 h for 2 days, followed by adjustments to maintain target serum concentrations at 5–8 ng/mL over 6 months), compared with baseline.[43] In patients with rheumatoid arthritis, everolimus (6 mg/day orally, for 12 weeks) was associated with modest increases in the concentrations of total cholesterol, LDL cholesterol, and triglycerides, which returned to baseline after the 12-week treatment.[44]

The overall risk of bias was classified as high for four articles,[30,32,39,43] some concerns for 11 articles,[31,33,34,36-40,42,44,45] and low for four articles[26,27,35,41] (figure 4). The main sources of risk and concerns for

Low risk
Some concerns
High risk

Intention to treat	D1	D2	D3	D4	D5	Overall
Boni et al (2012)[29]	+	!	+	!	+	!
Bruyn et al (2008)[44]	+	+	+	!	+	!
Chung et al (2019)[30]	!	+	–	–	–	–
Dickinson et al (2013)[31]	!	!	+	!	+	!
Drummond et al (2009)[32]	–	!	+	!	+	–
Gensler et al (2018)[38]	+	!	+	+	–	!
Gunderman et al (2014)[33]	!	!	+	+	+	!
Hörbelt et al (2020)[34]	–	+	+	+	–	!
Kraig et al (2018)[36]	!	!	+	–	!	!
Krebs et al (2007)[35]	+	+	+	+	+	+
Mannick et al (2021)[27]	+	+	+	+	+	+
Mannick et al (2014)[26]	+	+	+	+	!	+
Minturn et al (2021)[39]	–	!	+	!	+	–
Nussenblatt et al (2010)[40]	!	+	+	!	+	!
Palma et al (2022)[41]	+	+	+	+	+	+
Petrou et al (2014)[42]	!	!	+	!	+	!
Seyfarth et al (2013)[43]	!	!	+	–	–	–
Wen et al (2019)[45]	!	!	+	!	+	!
Per protocol						
Dugel et al (2012)[37]	!	–	+	!	+	!

Figure 4: **Assessments of the five domains of risk of bias in the included articles**
D1=randomisation process. D2=deviations from the intended interventions. D3=missing outcome data. D4=measurement of the outcome. D5=selection of the reported result.

bias arose from the selection of reported results (four articles with high risk of bias[30,34,38,43] and two with some concerns[36,48]), measurement of outcomes (three articles with high risk of bias[30,36,43] and nine with some concerns [31,32,37,39,40,42,44,45]), and randomisation processes (three articles with high risk of bias[32,34,39] and nine with some concerns[30,31,33,36,37,40,42,43,45]). Other sources of high risk of bias included deviations from intended interventions[37] and missing outcome data.[30]

On July 2, 2023, nine ongoing clinical trials registered on ClinicalTrials.gov were investigating the effects of rapamycin and its derivatives on ageing-related outcomes. The primary outcomes assessed in these trials were changes in phenotypic or functional biomarkers of ageing (walking speed, chair stand, standing, balance, grip strength, BMI, muscle mass, and waist circumference), visceral fat, systolic and diastolic heart function, aortic cross-sectional area, aortic distensibility, peripheral insulin sensitivity, glucose, albumin, carbon dioxide or bicarbonate concentrations, ovarian reserve, epigenetic markers, penetration of the blood–brain barrier by rapamycin, and cerebral glucose uptake (appendix pp 64–66).

Discussion

Rapamycin and its derivatives improved the immune, cardiovascular, and integumentary systems in healthy individuals or individuals with ageing-related diseases. Overall, these drugs had no significant effects on the endocrine, muscular, or neurological systems. The effects of rapamycin or its derivatives on the respiratory, digestive, renal, and reproductive systems were not assessed. There was no clear relationship between the dose of rapamycin or its derivatives and the effects of these drugs on different physiological systems. No serious adverse events were attributed to the interventions.

Although studies have reported that rapamycin and its derivatives can enhance learning and memory[49,50] and reduce neurodegeneration in animal models,[51] these effects were not observed in the human studies assessed in this systematic review.[36,41] Moreover, the reported effects on ageing-related macular changes were inconsistent. For instance, rapamycin reduced the need for anti-VEGF usage[40] but also led to the loss of visual acuity in individuals with geographic atrophy in age-related macular degeneration.[42]

In addition, in preclinical studies, pharmacological mTOR inhibition reduced age-related cardiac inflammation, fibrosis, hypertrophy, and systolic dysfunction.[52] Although improvements in the cardiac index and reductions in both pulmonary vascular resistance and pulmonary arterial pressure have been observed following intervention with everolimus,[43] the effects of rapamycin and its derivatives on cardiovascular parameters in humans should be assessed more comprehensively in future studies.

As individuals age, their glucose tolerance declines.[53] Overactivation of the mTOR pathway leads to the activation of S6K-1 and phosphorylated IRS-1, impairing the stimulation of PI3K by insulin and, subsequently, lowering insulin resistance in human muscle.[54] The effects of rapamycin on glucose metabolism differed by study setting. Although rapamycin treatment increased glucose turnover under conditions of induced peripheral hyperinsulinaemia,[35] it did not affect glucose turnover when there was low peripheral insulin,[35] and did not affect post-exercise insulin concentrations,[32] or post-amino acid infusion fasting insulin, C-peptide, glucose, or glucagon concentrations.[35]

mTOR is hypothesised to be a crucial regulator responsible for maintaining skeletal muscle mass.[55] Although animal studies investigating mTOR inhibition by rapamycin and its derivatives on the muscular system have reported inconsistent results,[56,57] the human studies we assessed reported no significant effects. However, in these studies, rapamycin was administered in single doses. Further research with different dosing regimens might be necessary to better understand the potential effects of rapamycin and its derivatives on the muscular system.

Topical rapamycin significantly reduced the expression of markers of skin ageing. However, the effects of systemically administered rapamycin or its derivatives on the skin were not investigated and, therefore, require further research.

As individuals age, their capacity to mount a robust immune response diminishes, rendering them more vulnerable to infections and poor response to vaccinations.[58] The mTOR pathway is a crucial signalling pathway within the immune system, controlling the activation, proliferation, differentiation, and function of immune cells.[59] Although rapamycin is known to be immunosuppressive, there are several mechanisms that might explain the immunostimulatory effect of rapamycin in vitro and in animal studies, such as the improvement of immune memory,[60] alteration of $CD8^+$ cell response,[61] and promotion of regulatory T-cell survival and function.[62] In the studies analysed in this Review, rapamycin and its derivatives improved immune function mainly by altering adaptive immunity. As individuals age, there is an accumulation of PD-1-positive T cells, which show reduced responsiveness to antigen stimulation.[63] This diminished response is attributable to the inhibitory effects of PD-1 on T cell-receptor-induced T-cell proliferation, cytokine production, and cytolytic function.[64] Everolimus decreased the numbers of PD-1 positive T cells in one human study[26] but not in another.[36] The lack of consistent positive outcomes across physiological systems could be attributed to small sample sizes, the low number of included studies, and the measurement of different outcomes within the same physiological systems across studies. Furthermore, only a few studies[26,27,35,41] were evaluated as having no risk of bias.

There was no clear relationship between the dose of rapamycin or its derivatives and the efficacy of these drugs in ameliorating ageing-related outcomes in the assessed studies. This finding suggests that even though the pharmacokinetics of rapamycin are well known, pharmacodynamic studies that focus on target (ie, mTOR) engagement and the effects of rapamycin on ageing-related biomarkers are needed to establish an adequate dosing regimen for geroprotection.

The use of rapamycin and its derivatives by organ transplant recipients and patients with cancer is associated with adverse events including infections, mouth ulcers, generalised pain, headache, fever, hypertension, nausea, abdominal pain, constipation, diarrhoea, urinary tract infection, peripheral oedema, anaemia, arthralgia, thrombocytopenia, hypercholesterolaemia, hypertriglyceridaemia, and increased creatinine.[65–67] Some of these adverse events were reported in the assessed studies. One study reported an increase in triglyceride concentrations,[43] which is consistent with the increase in triglyceride concentrations observed in kidney transplant recipients receiving rapamycin.[68] Although no serious adverse events were attributed directly to treatment with rapamycin or its derivatives in the studies on healthy individuals assessed here, it is important to note that in six studies, only single doses of rapamycin or its derivatives were given.[29,32,33,35,37,69] Although single doses of drugs can be used to study pharmacokinetic and pharmacodynamic characteristics, short-term tolerability, and safety, this approach is insufficient to confirm long-term safety. In a study where doses of 2–6 mg/day were given for 48 weeks, a significantly higher number of adverse events were reported in the treatment group than in the placebo group; specifically, infections were the most common adverse events,[41] in accordance with empirical data on the occurrence of bacterial infections in users of off-label rapamycin.[70] In addition, in the EXIST-3 study, which included everolimus-treated patients with tuberous sclerosis complex, aged 2–65 years, the core phase (18 weeks)[71] reported a tolerable safety profile and no deaths; however, the subsequent extended phase (48 weeks) of the same study reported two treatment-related deaths attributed to pneumonia and septic shock in younger patients (aged <6 years) after the data cutoff date.[72,73]

Five deaths due to serious adverse events suspected to be related to everolimus occurred in a study including postmenopausal women with breast cancer treated with everolimus (10 mg) and exemestane (25 mg) daily for 48 weeks or until disease progression, unacceptable toxicity, death, or discontinuation for any other reason; the causes of death were pneumonitis, bilateral pneumonia, and disease progression.[74] In another study, treatment-emergent adverse events resulting in permanent everolimus discontinuation occurred in 33% of individuals aged 65 years or older and 17% of those younger than 65 years.[75] These results stress the importance of assessing the long-term safety of such treatments in healthy individuals.

Studies in mammalian cells and yeast have reported the significant impact of rapamycin treatment on defects relating to microtubule functions (ie, spindle elongation, chromosome segregation, and nuclear migration) and chromosomal instability.[76,77] Additionally, negative regulation of STK38 (also known as NDR kinase) and STK CBK1 by TOR complex 1 in budding yeast raises questions about the implications of this regulatory pathway in higher eukaryotes, including humans.[78] Dysregulation of NDR kinases has been associated with various diseases, including cancer.[79] Therefore, inhibition of CBK1 by rapamycin could potentially lead to unforeseen adverse effects on cell cycle and physiology. Nevertheless, it is essential for future studies to provide more information on the occurrence and timing of adverse events, along with the blood concentrations of rapamycin and related compounds. These data would aid in establishing the targeted concentrations of rapamycin and its derivatives in blood, which is crucial for minimising the adverse effects of the geroprotective treatment.

In an online survey used to record the self-reported experiences of 333 adults with a history of off-label rapamycin use and 172 adults who had never used rapamycin,[7] 95% of participants reported that they were using rapamycin for "healthy longevity/anti-ageing". The most common dosing regimen was 6 mg once per week and 6 mg once every 14 days, respectively. Some participants reported taking high doses of rapamycin (20 mg/week).[70] No serious adverse events were reported in this study. However, it is possible that participants who experienced serious adverse events did not participate in this survey.

Rapamycin and its derivatives showed positive effects on the immunological, cardiovascular, and integumentary systems. However, the overall small number of studies and underassessment of the respiratory, digestive, renal, and reproductive systems, along with the scarcity of long-term studies, underscore the need for further exploration of the effects of rapamycin and its derivatives.

Contributors

All authors contributed equally to conceptualisation, data curation, formal analysis, investigation, methodology, validation, visualisation, the writing of the original draft, and the review and editing of the manuscript. DJWL and AHK also did project administration and used the specified software to organise and manage the articles and assess the risk of bias. ABM supervised the study.

Declaration of interests

We declare no competing interests.

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