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Review

Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island

Sebastian I. Arriola Apelo and Dudley W. Lamming

Department of Medicine, University of Wisconsin–Madison and William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin.

Address correspondence to Dudley W. Lamming, PhD, William S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Room C3127 Research 151, Madison, WI 53705. Email: dlamming@medicine.wisc.edu

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Abstract

Rapamycin (sirolimus) is a macrolide immunosuppressant that inhibits the mechanistic target of rapamycin (mTOR) protein kinase and extends lifespan in model organisms including mice. Although rapamycin is an FDA-approved drug for select indications, a diverse set of negative side effects may preclude its wide-scale deployment as an antiaging therapy. mTOR forms two different protein complexes, mTORC1 and mTORC2; the former is acutely sensitive to rapamycin whereas the latter is only chronically sensitive to rapamycin in vivo. Over the past decade, it has become clear that although genetic and pharmacological inhibition of mTORC1 extends lifespan and delays aging, inhibition of mTORC2 has negative effects on mammalian health and longevity and is responsible for many of the negative side effects of rapamycin. In this review, we discuss recent advances in understanding the molecular and physiological effects of rapamycin treatment, and we discuss how the use of alternative rapamycin treatment regimens or rapamycin analogs has the potential to mitigate the deleterious side effects of rapamycin treatment by more specifically targeting mTORC1. Although the side effects of rapamycin are still of significant concern, rapid progress is being made in realizing the revolutionary potential of rapamycin-based therapies for the treatment of diseases of aging.

Keywords: Intermittent rapamycin—Lifespan—Mouse—Rapamycin analogs

Rapamycin is a macrolide produced by the bacterium *Streptomyces hygroscopicus* and first discovered in soil samples from Easter island (1). Although rapamycin was originally described as an antifungal agent, it was soon discovered that rapamycin has immunosuppressant activity, inhibiting T-cell proliferation (2,3), and that it suppressed incorporation of amino acids into cellular proteins, inhibiting protein translation (4). Rapamycin is structurally similar to the immunosuppressant macrolide FK506, which inhibits calcineurin activity and IL-2 production in T cells (5–7). Several studies performed comparative analyzes of rapamycin and FK506 mechanism of action, leading to the identification cloning of a common binding protein (FKBP12). The same year that the structure of FKBP12 was discovered, the genes targeted by the rapamycin–FKBP complex (Tor1 and Tor2), which encode for the protein TOR, were identified in yeast, and soon afterwards its mammalian homologue, the mechanistic target of rapamycin (mTOR) was isolated (reviewed in (8)). Over the last 20 years—particularly in the last decade—our understanding of the diverse set of cellular functions and substrates regulated by this kinase has grown by leaps and bounds. It has become clear that mTOR is sensitive to many varied environmental and endocrine stimuli and that mTOR is a central regulator not only of growth and proliferation but also of metabolism and even aging.

mTOR is a serine/threonine protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)–related kinase family and is found in two protein complexes (mTORC1 and mTORC2) with distinct protein components and substrates (9). mTORC1 is acutely sensitive to environmental stimuli, most famously amino acids but also glucose and oxygen, whereas mTORC2 is best characterized as an effector of insulin/IGF-1 signaling downstream of PI3K (reviewed in (10–12)). mTORC1 controls protein translation, autophagy, and many other cellular processes through the phosphorylation of substrates that include S6K, 4E-BP1, and ULK1, whereas mTORC2 is required for maximal activation of numerous kinases, including AKT. Although a full discussion of how mTOR signaling coordinates cellular processes with the availability of nutrients and hormonal signaling is beyond the scope of this review, it is worth noting that the past year has seen significant advances in our understanding of how mTORC1 activity is regulated by amino acids at

the molecular level. Sestrin2 was identified as a leucine-binding protein and CASTOR1/2 as arginine-binding proteins which regulate mTORC1 localization to the lysosome; SLC38A9 was identified as a lysosomal arginine transporter that signals arginine sufficiency to mTORC1 via Ragulator (13–16).

A key difference between the two mTOR complexes is their sensitivity to rapamycin (Figure 1); whereas mTORC1 is acutely sensitive to rapamycin, mTORC2 is comparatively insensitive to rapamycin, and prolonged, chronic exposure to the drug is required to disrupt mTORC2 in vivo or in cell culture (17,18). The sensitivity of mTORC2 to rapamycin varies by cell line and tissue type, with mTORC2 in liver, adipose tissue, and muscle being sensitive to chronic exposure to rapamycin, but with mTORC2 in other tissues (eg, thymus, kidney, and stomach) being completely resistant to rapamycin (18,19). The differential sensitivity of each mTOR complex to rapamycin is of major relevance for aging research because, as discussed in this review, it may determine the balance between the prolongevity benefits and negative side effects of the drug.

Rapamycin: The Antiaging Molecule

Short after the discovery of rapamycin, studies identified its capacity to inhibit cancer cell proliferation in mouse models, while parallel studies explored the potential of rapamycin as an immunosuppressant for organ transplants (20,21). However, it was not until the discovery of the TOR pathway as an important regulator of aging in yeast and *Caenorhabditis elegans* that rapamycin was first considered as a potential antiaging therapy (22,23). Since these initial studies, rapamycin has been reported to extend lifespan in yeast, worms, and flies (24–28). Rapamycin has also shown efficacy against mouse models of age-related diseases, including cancer and neurodegenerative diseases including Alzheimer's disease, ameliorates age-related cognitive decline, and can even rejuvenate the hearts of aged mice (29–35).

Rapamycin was first shown to extend the lifespan of wild-type mice in 2009 by the National Institute on Aging Interventions Testing Program, and since that time, numerous studies in mice of different genetic backgrounds by many independent laboratories have confirmed the strong prolongevity effect of rapamycin (Table 1). However, significant discussion has surrounded the mechanism of action by which rapamycin promotes lifespan and whether it is a true antiaging intervention or "merely" a potent anticancer agent.

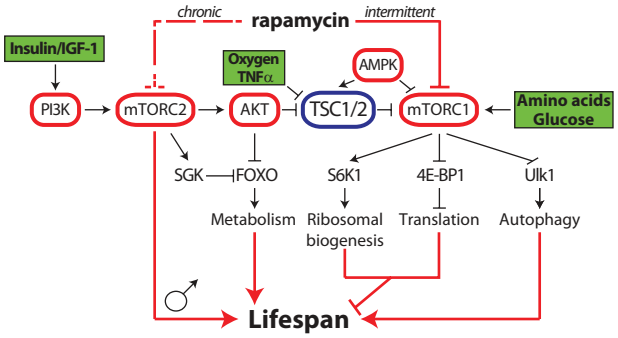

Figure 1. Theoretical model of rapamycin regulation of lifespan by mTOR signaling. Rapamycin acutely inhibits mTORC1, while chronic administration also inhibits mTORC2 in the majority of tissues. Intermittent administration of rapamycin in the form of single acute doses (eg, 2 mg/kg of rapamycin every 5 days) more precisely targets mTORC1 (45). Repression of mTORC1 promotes longevity through pathways that likely include the inhibition of S6K1, protein translation, and increased autophagy. Conversely, mTORC2 inhibition results in metabolic dysfunction and decreases the lifespan of male mice through an as yet undetermined mechanism.

Rapamycin is certainly a potent anticancer agent in mice as well as in certain human cancers (36,37). The possibility that the effect of rapamycin on longevity is primarily due to delaying or slowing cancers was recently argued by Neff and colleagues, who performed a large-scale analysis of more than 150 aging-related traits in more than 25 different tissues of C57BL/6J rapamycin-treated male mice (38). They discovered that rapamycin only improved a subset of the observed traits, whereas another subset of traits was actually worsened by rapamycin treatment. The authors argue that the putatively age-related traits improved by rapamycin are similarly affected in young and old mice, suggesting that rapamycin may improve certain health-related phenotypes but that these effects are independent of any effect on aging (38).

However, many other studies now support the idea that rapamycin is a true antiaging intervention. Even a relatively brief treatment with rapamycin significantly decreases the mortality rate of wild-type C57BL/6J mice (39), and rapamycin has a robust effect on not only average lifespan but also maximum lifespan of genetically heterogeneous mice (40–42). In addition, a variety of age-related pathologies are delayed by rapamycin treatment, including age-related atypical myocardium nuclei, liver steatosis, and decline in voluntary activity (33), as well as attenuating age-related changes in the extracellular matrix of muscle tendon that lead to an age-associated increase in tendon stiffness (43). In aged rats and mice, rapamycin acts to decrease body weight and adiposity; interestingly, in aged rats but not in aged mice, this was accompanied by a decrease in leptin (42,44,45). Rapamycin also acts to slow intestinal aging in Drosophila, while promoting the self-renewal of intestinal stem cells in mice (46,47). Finally and most dramatically, rapamycin has been reported to actually reverse aging, rejuvenating tissues including the aging heart as well as hematopoietic stem cells (31,39).

Genetic mouse models of decreased mTORC1 signaling support the idea that mTORC1 signaling promotes aging. Mice lacking either the mTORC1 substrate *S6K1*, or mice doubly heterozygous for *mTOR* and *mLST8*, show a significant increase in female lifespan (17,48). Mice expressing a hypomorphic allele of mTOR have a significant increase in both male and female lifespan, exhibit improved biomarkers of tissue aging in kidney, liver, and brain, and also display improved spatial learning, memory, co-ordination, and muscle strength (49). Inhibition of hepatic mTORC1 by liver-specific deletion of *Raptor* rescues an age-associated defect in ketogenesis (50), whereas constitutive activation of 4E-BP1, a substrate normally repressed by mTORC1, specifically in skeletal muscle preserves metabolic health during aging (51). Finally, in line with these results, two long-lived mouse models—Snell dwarf mice and mice lacking the growth hormone receptor—have significantly decreased mTORC1 signaling (52). Genetic mouse models of mTORC1 inhibition thereby add support to the concept that rapamycin is a true antiaging intervention.

Side Effects of Rapamycin Pose a Barrier to its Wide-scale Use

Rapamycin (Sirolimus) and its FDA-approved analogs Everolimus (Afinitor) and Temsirolimus (Torisel) have been extensively used in humans as immunosuppressants following kidney and liver transplants, to treat certain types of cancers, and for the treatment of complications of tuberous sclerosis, a genetic disorder leading to aberrant activation of mTORC1 (53). A diverse and severe set of

Table 1. The Effect of Rapamycin on Mouse Lifespan

Strain	Sex	Starting Age	Rapa Dose	Route	Control (days)	Δ Lifespan (%)	Reference
Wild-type mice							
UM-HET3	Male	20 months	14 ppm	Diet	—	9	(40)
UM-HET3	Female	20 months	14 ppm	Diet	881–895	14	(40)
C57BL/6J.Nia	MF	22–24 months	4 mg/kg	IP 1×/2 days	~795	>14[a]	(39)
UM-HET3	Female	9 months	14 ppm	Diet	843–891	18	(41)
UM-HET3	Male	9 months	14 ppm	Diet	780–851	10	(41)
129/Sv	Female	2 months	1.5 mg/kg	SC 3×/week 2 weeks per 4	759	10	(93)
C57BL/6J.Rj	Male	4, 13, or 20 months	14 ppm	Diet	~900	~10[a]	(38)
UM-HET3	Male	9 months	4.7 ppm	Diet	807	3[NS]	(42)
UM-HET3	Male	9 months	14 ppm	Diet	807	13	(42)
UM-HET3	Male	9 months	42 ppm	Diet	807	23	(42)
UM-HET3	Female	9 months	4.7 ppm	Diet	896	16	(42)
UM-HET3	Female	9 months	14 ppm	Diet	896	21	(42)
UM-HET3	Female	9 months	14 ppm	Diet	896	26	(42)
C57BL/6J.Nia	Male	4 months	14 ppm	Diet	806*	11*	(114)
C57BL/6J.Nia	Female	4 months	14 ppm	Diet	826*	16*	(114)
C57BL/6J.Nia	Female	20 months	2 mg/kg	IP 1×/5 days	897	7	(45)
Disease models							
Pten$^{-/-}$	MF	1 month	10 mg/kg (Everolimus)	Oral	66*	>292*[a]	(115)
FVB/N HER-2/neu	Female	2 months	1.5 mg/kg	SC 3×/week 2 weeks per 4	288	13.6	(116)
SOD1$^{H46R/HR8Q}$	MF	1.5 months	14 ppm	Diet	232	*NS*	(117)
p53$^{+/-}$	Male	<5 months	1.5 mg/kg	Water	373*	28*	(118)
p53$^{+/-}$	Male	>5 months	1.5 mg/kg	Water	373*	10*	(118)
p53$^{-/-}$	Male	2 months	0.5 mg/kg	Oral 1×/day 5 days on/9 days off	161	35	(119)
Lmna$^{-/-}$	MF	1 month	14 ppm	Diet	46	35	(120)
Lmna$^{-/-}$	MF	1 month	8 mg/kg	IP 1×/2 days	55	56	(120)
Rb1$^{+/-}$	Male	2 months	14 ppm	Diet	369	13.8	(121)
Rb1$^{+/-}$	Female	2 months	14 ppm	Diet	378	8.9	(121)
Bmal1$^{-/-}$	MF	16 weeks	0.5 mg/kg	Water	~240	47	(122)
HER-2/neu	Female	2, 4, or 5 months	0.45 mg/kg	SC 3×/week 2 weeks per 4	282, 278, 289	5.7[NS], 6.1, 5.5	(123)
C57BL/6NCr HFD	Male	12 months	1.5 mg/kg	IP 1×/week	684	b	(99)
Ndufs4$^{-/-}$	MF	<1 month	42 ppm	Diet	52	29[NS]	(124)
Ndufs4$^{-/-}$	MF	<1 month	378 ppm	Diet	52	92	(124)
Ndufs4$^{-/-}$	MF	<1 month	8 mg/kg	IP 1×/day	52	119	(124)
Rag2$^{-/-}$	MF	3 months	14 ppm	Diet	310	121	(125)
IFN-γ$^{-/-}$	MF	5 months	14 ppm	Diet	398	34	(125)
C57BLKS/J *lepr*$^{db/db}$	Male	4 months	14 ppm	Diet	349	−16	(126)
C57BLKS/J *lepr*$^{db/db}$	Female	4 months	14 ppm	Diet	487	−18	(126)

Notes: The table shows the impact of rapamycin on median lifespan in mouse studies since 2009 where longevity or mortality rate was determined. Sex is listed separately for males and females where sex-specific data exist. The rapamycin dosage listed for dietary administration indicates the drug concentration in the ad libitum fed diet; the dosage listed for administration in water, or administered intraperitoneally (IP) or subcutaneously (SC), indicates the dosage in milligram per kilogram of body weight. Control indicates median lifespan of control group in days; Δ lifespan is the change in median lifespan (* indicates that mean is reported instead). MF indicates that the lifespan results were not broken down by sex or that sex was not reported.

[a]Lifespan study % increase was not determined.

[b]100% of rapamycin-treated mice survived to 2 years of age vs 40% of control mice.

[NS]Not statistically significant.

Control lifespan and percentage change are estimated when precise information is not listed in the referenced study.

negative side effects likely preclude the wide-scale use of rapamycin and its analogs as a prolongevity agent. These include hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia, glucose intolerance, insulin resistance and new-onset diabetes, anemia and thrombocytopenia, dermatological events, gastrointestinal disorders, sinusitis, respiratory and urinary infections, and testicular dysfunction (54–61). Although many of these effects seem to be reversible—particularly the dermatological and testicular side effects—the immunological consequences are extremely serious, occasionally resulting in death from infections (61).

An understanding of the mechanistic basis for many of these side effects has been elusive and was significantly delayed by the perception that most effects of rapamycin in vivo were mediated by mTORC1. Over the last several years, it has instead become apparent that chronic treatment with rapamycin also inhibits mTORC2 in vivo in the majority of tissues, including liver, adipose tissue, skeletal muscle, and immune cells (17,19,62). It has now been conclusively demonstrated that disruption of mTORC2 contributes to several of these effects, particularly glucose intolerance, diabetes, and immunosuppression.

Glucose Intolerance and Diabetes

It has been known for some time that rapamycin treatment can lead to the development of glucose intolerance in both humans and rodents (63–65). However, the molecular and physiological mechanisms that mediated this effect were unclear, with most attention focusing on the negative impact of rapamycin on pancreatic beta cells, an effect associated with decreased mTORC1 signaling (66,67). Several years ago, it was discovered that rapamycin induces hepatic insulin resistance, an effect mediated by disruption of mTORC2 (17). Using a mice conditionally expressing *Rictor*, an essential component of mTORC2, it was determined that mTORC2 in the liver is a key regulator of glucose metabolism and is essential for the insulin-mediated suppression of hepatic gluconeogenesis (17,68).

Research from other laboratories suggests that mTORC2 in many tissues, including skeletal muscle, adipose tissue, and the pancreatic beta cell, may all play a role in the regulation of glucose tolerance. Genetic disruption of mTORC2 in adipose tissue causes pronounced metabolic effects, including insulin resistance, hyperinsulinemia, and after 9 months of age, glucose intolerance (69–71). mTORC2 disruption in skeletal muscle results in impaired insulin-stimulated glucose uptake and glycogen synthase activity, which correspond with observed mild glucose intolerance (72,73). Genetic disruption of mTORC2 in beta cells, also achieved through tissue-specific deletion of *Rictor*, likewise results in hyperglycemia and glucose intolerance, resulting from a reduction in pancreatic insulin and reduced insulin secretion in response to glucose (74).

Although inhibition of mTORC2 in all of these tissues likely contributes to the metabolic effects of rapamycin, a role for mTORC2 in the brain in metabolism was recently discovered. Mice in which *Rictor* was deleted in neurons through the use of *Nestin-Cre* are glucose intolerant and insulin resistant and also have increased adiposity (75). The increased adiposity of these mice is explained by the combination of increased calorie intake per unit of lean mass, reduced energy expenditure, and lower body temperature. Intriguingly, deletion of *Rictor* specifically in the POMC neurons of the hypothalamus causes a very similar phenotype, with increased fat deposition and impaired glucose tolerance (75). Although previous research strongly suggested that hypothalamic mTORC1 regulates energy homeostasis, it was recently shown that the hypothalamic expression of DEPTOR, a protein which interacts with both mTORC1 and mTORC2, is regulated by diet and feeding status (76). Taken together, these data suggest that hypothalamic mTORC2 may have a major role on whole body lipid and glucose metabolism and energy balance and that inhibition of hypothalamic mTORC2 by rapamycin—if it indeed occurs—may have major metabolic consequences.

An important consideration in the use of rapamycin for chronic diseases may be the metabolic adaptation to prolonged mTOR inhibition. Although short-term studies with rapamycin in mice have largely reported metabolic dysfunction as discussed earlier, at least one longer-term study reports metabolic adaptation and improved insulin sensitivity (as assessed by insulin tolerance test) following 5 months of rapamycin administration (77). Notably, glucose intolerance persisted throughout the course of rapamycin administration in this study. The effect of rapamycin on gene expression can also vary based on length of treatment, with genes initially elevated by short-term rapamycin administration being repressed during long-term rapamycin administration (78). These results suggest that the physiological and molecular mechanisms which mediate the organismal phenotypes of rapamycin-treated animals may shift over time; understanding the significance of these results to the extension of lifespan by rapamycin will require significant additional study.

Immunosuppression

The mechanistic basis for the potent immunosuppressive effects of rapamycin was originally assumed to be due to an antiproliferative effect on T cells, but it is now believed that rapamycin primarily impacts immunity through nonproliferative mechanisms (reviewed in (62)). One major pathway through which rapamycin exerts its immunosuppressive effects is promoting an increase in immunosuppressive T-regulatory cells (Tregs); interestingly, both mTOR complexes play a role in the regulation of Tregs, with mTORC2 activity normally suppressing Treg differentiation (62,79). However, recently it has become clear that although the effects of rapamycin on Tregs are extremely important, rapamycin exerts its effects upon a wide range of immune cell types, including both $CD4^+$ and $CD8^+$ T cells, B cells, and macrophages (62,79–83).

Rapamycin and Sexual Dimorphism

Consistently, rapamycin has shown to benefit females more than males, in both UM-HET3 genetically heterogeneous mice and inbred C57BL/6J mice, and independent of starting treatment when young or old (Table 1). One possible explanation for this is sex-specific differences in rapamycin uptake or metabolism (42). In support of this hypothesis, female UM-HET3 mice tend to have higher blood levels of rapamycin than UM-HET3 males when both sexes are provided rapamycin at equal concentration in the diet, and differences in the effect of rapamycin on lifespan are minimized when rapamycin levels are raised to 42 ppm (42). However, it is not clear that there is a significant difference in blood levels of rapamycin between male and female mice at a lower dosage where the sexual dimorphism is most apparent. As of yet, there has not been a sufficiently detailed and comprehensive analysis of rapamycin concentrations over time in the blood or tissues of mice to fully evaluate how sex-specific differences in drug uptake and metabolism may contribute to the sexually dimorphic impact of rapamycin on lifespan.

The hypothesis that sex-specific differences in drug uptake and metabolism explain the differential effect of rapamycin on male and female lifespan is also not a fully satisfying explanation in light of the similar, sexually dimorphic impact of genetic interventions in the insulin/IGF-1/Akt/mTOR signaling pathway on mouse lifespan. Mouse models in which there is a greater benefit to female lifespan than male lifespan include mice lacking either *Irs1* or *S6K1* and mice doubly heterozygous for *mTOR* and *mLST8* (17,48,84). Notably, a calorie-restricted diet, the gold standard for age-related interventions and one that has been proposed to promote longevity in part via reduced insulin/IGF-1/mTOR pathway signaling, also benefits female mice more than males (85–87).

Recent observations suggest several other possible explanations for the differential efficacy of rapamycin on male and female longevity. First, as discussed earlier, Rictor, a key component of mTORC2, is critical for the survival of male mice, but depletion of Rictor has no effect on female longevity (88). Thus, inhibition of mTORC2 signaling by rapamycin, and its negative effects on males, might explain the sexually dimorphic benefit. However, as noted, the sexually dimorphic effect of rapamycin on lifespan is minimized when a high dosage of rapamycin is used, and no sexual dimorphism in longevity is observed in mice expressing very low levels of mTOR (42,49), making an mTORC2-based explanation more complicated. In support of this model, two particularly long-lived mouse strains—Snell dwarf mice and mice lacking the growth hormone receptor—not only have significantly decreased mTORC1 signaling but also have increased mTORC2 signaling (52). A second, possibly simpler

explanation is suggested by the recent observation that mTORC1 signaling is naturally higher in the liver and heart of young female mice as compared with age-matched males (89). If mTORC1 signaling in these tissues is critical to longevity, and is normally higher in females, this could potentially explain the greater benefit of rapamycin to females.

A third theory is proposed in this issue by Lind and colleagues, who suggest that the sexual dimorphism of rapamycin is explained by the fitness cost of lifespan extension, with the smaller sex paying less fitness cost while enjoying further lifespan extension (90). As males are larger than females in most mammalian species, interventions in the insulin/IGF-1/mTOR signaling might therefore generally be expected to benefit females more than males. Notably, this evolutionarily-based theory does not exclude sex-specific differences in mTORC1 or mTORC2 signaling as molecular mechanisms underlying the differential response to rapamycin. Additional research, potentially in mammalian species or inbred strains with female-biased size dimorphism, will be required to test this intriguing hypothesis.

Intermittent Rapamycin

Since the initial discovery of the potent effects of rapamycin on longevity, it has been suggested that intermittent administration of rapamycin might be a potential method to limit some of the negative side effects of rapamycin (91,92), although the term "intermittent" has been utilized to encompass a number of different dosing strategies. One such strategy—rapamycin administered three times per week for 2 weeks, followed by a 2-week drug holiday—significantly extends the lifespan of 129/Sv female mice (93). Also in this issue, Scarpace and colleagues report that rapamycin administered three times a week for 5 weeks reduces adiposity and leptin synthesis, improving the lean/fat ratio and normalizing plasma leptin levels in old rats (94). The authors suggest that this routine more specifically targets peripheral tissues (adipose), which communicate with the hypothalamus to trigger the observed anorexic response.

However, the negative effects of chronic rapamycin on glucose homeostasis persist for at least 2 weeks following cessation of treatment (95,96), and so a three time per week schedule—with or without a washout period—may therefore be of limited use in reducing side effects. Notably, the washout period for the effects of rapamycin with respect to other side effects including immunosuppression and hyperlipidemia has not been established in mice. In humans, while sirolimus has a relatively short initial half-life, the terminal half-life has been reported to be 80 hours (97); it is likely that a single 10-mg dose of sirolimus remains in the circulation at or above 1 ng/mL for a minimum of a week.

The discovery that many of the negative side effects of rapamycin are mediated by mTORC2, and the prolonged exposure of mice to rapamycin required to inhibit mTORC2, suggested that a dosing regimen consisting of single doses of rapamycin given at sufficiently lengthy intervals such that only mTORC1 is significantly inhibited could extend lifespan with a minimum of side effects. Single doses of 2-mg/kg rapamycin injected once every 5 days was the most frequent treatment regimen that did not impair glucose homeostasis; this dosing regimen also had a reduced impact on the immune system while still significantly inhibiting mTORC1 in many tissues (98). A more spaced schedule (weekly treatment) has also been reported to reduce side effects, although in the context of a high-fat diet, glucose tolerance was still decreased (99). This treatment regimen increased the survival of female mice on a high-fat diet at 2 years of age, but lifespan was not determined, and the effect of such a relaxed schedule on the longevity of nonmetabolically challenged mice remained open.

In this issue, we report that the intraperitoneal administration of 2-mg/kg rapamycin once every 5 days—the same regimen recently demonstrated to reduce the metabolic and immunological side effects of rapamycin—to aged female C57BL/6J.Nia mice significantly increases lifespan as well as maximum lifespan (45). Interestingly, the increase in lifespan we observed following intermittent intraperitoneal administration of rapamycin was greater than that in a recent study of similarly aged female C57BL/6J.Nia mice fed 14 ppm of encapsulated rapamycin in the diet (100). A question for further study is whether the route of administration impacts lifespan, possibly due to different peak and trough concentrations of rapamycin. An alternative hypothesis, which we favor, is that inhibition of mTORC2 by chronic dietary administration limits the benefits achieved via rapamycin-mediated inhibition of mTORC1. Our findings suggest that a therapeutic window for rapamycin may exist in which the beneficial effects of rapamycin of aging and age-related diseases mediated by mTORC1 inhibition can be realized while minimizing mTORC2-related side effects.

Rapalogs

In this review, we have focused on rapamycin (sirolimus) due to the extensive use of this compound in aging research. However, several rapamycin analogs (rapalogs) have been developed to improve the pharmacokinetics of the drug, and therefore increase efficacy and specificity. Rapalogs include the FDA-approved everolimus and temsirolimus plus ridaforolimus, zotarolimus, and 32-deoxo-rapamycin (101). Everolimus and temsirolimus are FDA approved, and have been extensively trialed for various types of cancer, and the efficacy of rapalogs in the clinical setting has been extensively reviewed (102,103). However, little is known about the comparative levels of side effects of these compounds.

A comparative analysis between the three FDA-approved mTOR inhibitors sirolimus, everolimus, and temsirolimus was recently performed in order to specifically determine the effect of each compound on glucose homeostasis and the immune system in mice. Compared with rapamycin, both everolimus and temsirolimus showed a reduced but still significant impairment of glucose and pyruvate tolerance; all three compounds had similar impacts on splenocyte number (98). One possible explanation for the reduced metabolic impact of everolimus is that everolimus has an approximately 50% shorter blood half-life than sirolimus (104), resulting in lower chronic levels that could inhibit hepatic mTORC2.

Although these results suggest that everolimus and temsirolimus might have reduced side effects relative to sirolimus, these compounds are still not risk free. In human clinical trials, both everolimus and temsirolimus significantly increased the incidence of hyperglycemia and increased median fasting glucose by almost 70% (105), and everolimus reduced muscle and tumor glucose uptake in patients with metastatic insulinoma, suggesting an effect on insulin resistance (106). A recent study of everolimus in children and adolescents with tuberous sclerosis, a genetic disease which results in increased mTORC1 signaling, observed metabolic abnormalities including hypercholesterolemia and hypertriglyceridemia in more than two thirds of patients and a significant number of infections, including one case of death resulting from *Escherichia coli* sepsis (61).

The dose, however, makes the poison; the children and adolescents receiving everolimus for the treatment of tuberous sclerosis

received up to 7.5 mg of everolimus per day for a median time of 15 months (61). In contrast, a recent study conducted by Novartis used a much lower dosage, in which elderly adult patients received placebo, 0.5 mg/day, 5 mg/week, or 20 mg/week of everolimus for 6 weeks (107). Following cessation of everolimus, patients received a flu vaccination, and patients receiving everolimus had a substantially improved immune response; everolimus "rejuvenated" the immune system. Notably, this everolimus regimen was relatively well tolerated, with a severe everolimus-related adverse result—mouth ulcers—in only a single patient receiving the highest dosage of the drug.

It is not possible to say at this time that the side effects can be eliminated by the use of rapamycin analogs or altered dosing regiments. Indeed, even at the lowest dosage used by Mannick and colleagues, more than 40% of participants experienced at least one treatment-related adverse event, compared with 20% of those receiving a placebo (107).These findings suggest that, as with intermittent rapamycin in mice, a therapeutic window for rapamycin analogs for the treatment of certain age-related conditions may also exist in humans.

Conclusions

It has been known for several years that rapamycin extends lifespan of model organisms including mice of several genetic backgrounds. Less consensus has been reached on the idea that rapamycin also delays the aging process, rather than only preventing or slowing cancer development. However, it is not this argument that has prevented a generic use of rapamycin as antiaging therapy in humans, but rather, a diverse and severe set of side effects, including, but not limited to, impaired lipid and glucose metabolism and immune suppression. In recent years, it has become clear that many of the side effects caused by chronic rapamycin treatment are due to in vivo disruption of mTORC2 in most metabolically active tissues, including the liver, muscle, and adipose tissue. This discovery has led to the development of alternative treatment regimens aiming to selectively inhibit mTORC1. Intermittent rapamycin administration is one of those alternatives that has been widely investigated, and in this issue, we show that such a regimen is able to robustly extend mouse lifespan while minimizing several of the most severe side effects; rapamycin analogs with a reduced effect on glucose metabolism may also be promising candidates for further development and testing as antiaging compounds.

Although many challenges remain to translating the phenomenal benefits of rapamycin-based therapies into routine clinical use for age-related diseases, the future for rapamycin-based therapies is bright. Many of the questions surrounding the use of rapamycin for age-related diseases outside of the laboratory will be addressed over the next several years through studies of rapamycin in companion animals (108,109). The exposure of companion animals to natural environmental pathogens is one of the most critical questions to address; although few studies on the side effects of rapamycin or analogs in companion animals have been published, a recent small-scale study of everolimus in canines suggests that high, immunosuppressive doses of rapamycin are associated with an increased risk of infection (110). It remains to be seen whether much lower levels of rapamycin will have negative consequences on canine immunity. The metabolic side effects of rapamycin may also be of concern; hyperinsulinemia as a result of increased glucose-stimulated insulin secretion has been reported in dogs treated with rapamycin (111). Finally, companion animals are not climate controlled to the same degree as laboratory rodents; it was only recently discovered that rapamycin impairs cold tolerance (112), and thus there is the possibility that rapamycin may have effects which vary based on climate. There is already keen interest by drug companies in developing compounds that exclusively regulate mTORC1 (113), which will further spur advances in translating the potential benefits of rapamycin and related compounds into the clinic. In combination with further mechanistic understanding of how signaling downstream of mTORC1 and mTORC2 regulate aging as well as side effects, and additional studies to thoroughly understand the therapeutic window for rapamycin, the next few years will significantly increase our understanding of the practicality of treating humans for age-related diseases with rapamycin, and perhaps see the development of new compounds that bypass many of the negative side effects of rapamycin.

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