Available online at www.sciencedirect.com

ScienceDirect

Journal of Genetics and Genomics 41 (2014) 459−463

JGG

PERSPECTIVE

Rapamycin and Ageing: When, for How Long, and How Much?

Matt Kaeberlein*

Department of Pathology, University of Washington, Seattle, WA 98195-7470, USA

Received 28 February 2014; revised 18 June 2014; accepted 28 June 2014

Available online 7 August 2014

The mechanistic target of rapamycin (mTOR) is a nutrient and growth factor responsive kinase that modulates lifespan in species from yeast to mice (Johnson et al., 2013b). mTOR exists in two complexes within cells, mTOR complex I (mTORC1) and mTOR complex 2 (mTORC2) (Laplante and Sabatini, 2012). Abundant evidence suggests that mTORC1 is the primary mTOR complex involved in regulating longevity: mutations that reduce the activity of mTORC1 have been shown to extend lifespan in yeast (Kaeberlein et al., 2005; Powers et al., 2006), nematode worms (Vellai et al., 2003; Jia et al., 2004), fruit flies (Kapahi et al., 2004), and mice (Lamming et al., 2012), as has deletion of the mTORC1 substrate ribosomal S6 kinase (Fabrizio et al., 2001, 2004; Kapahi et al., 2004; Pan et al., 2007; Selman et al., 2009). Consistent with these genetic data, treatment with the mTORC1 inhibitor rapamycin has also been found to increase lifespan in yeast (Powers et al., 2006; Medvedik et al., 2007), worms (Robida-Stubbs et al., 2012), fruit flies (Bjedov et al., 2010), and mice (Harrison et al., 2009).

mTOR inhibition is believed to play a central role in mediating the beneficial effects of dietary restriction (DR) on healthy ageing (Kapahi and Zid, 2004; Kennedy et al., 2007). DR, which can be defined as a reduction in nutrient availability in the absence of malnutrition, is the most studied intervention for extending lifespan and enhancing healthy ageing across a diverse range of model organisms (Masoro, 2005; Anderson and Weindruch, 2012). DR is sufficient to reduce mTORC1 activity in each of the organisms where it has been shown to increase lifespan, and epistasis studies have placed DR in the same genetic pathway as mTORC1 with respect to lifespan in yeast (Kaeberlein et al., 2005; Steffen et al., 2008), nematodes (Ching et al., 2010), and fruit flies (Kapahi et al., 2004; Zid et al., 2009). These observations, along with the fact that mTORC1 inhibition is sufficient to extend lifespan in each of these species, has led to the general consensus that inhibition of mTORC1 plays a direct role in promoting longevity and healthspan in response to DR (Kapahi et al., 2010; Kaeberlein, 2013a).

As of early 2014, at least seven independent studies have reported lifespan extension from rapamycin in wild type mice (Table 1), with most studies using a dietary formulation where rapamycin is encapsulated for enteric release (Nadon et al., 2008). The first report, published in 2009, demonstrated that UMHET3 mice fed a diet containing encapsulated rapamycin at 14 ppm (~2.24 mg/kg/day) beginning at 600 days of age is sufficient to increase lifespan in both male and female animals (Harrison et al., 2009). Subsequent reports where rapamycin feeding was initiated in young adulthood showed a similar magnitude of lifespan extension in UMHET3 mice (Miller et al., 2011). Rapamycin feeding has also been shown to extend lifespan in C57BL/6J mice when initiated at mixed ages (Neff et al., 2013) or as late as 19 months of age in C57BL/6N mice (Zhang et al., 2014). Recently, a partial dose response study was performed in UMHET3 mice treated with either 4.7, 14, or 42 ppm rapamycin in the diet, with the striking result that animals fed the highest dose of rapamycin lived the longest (Miller et al., 2014). Thus, it seems likely that all of the prior studies examining effects of rapamycin on lifespan and age-related health measures have been performed at doses of the drug that are sub-optimal for longevity.

In addition to the partial dose response study reported by the National Institute on Ageing Interventions Testing Program (ITP) in UMHET3 mice (Miller et al., 2014), one additional study suggests that even higher doses of rapamycin could result in greater improvements in longevity and healthspan than have thus far been observed (Chen et al., 2009). Treating C57BL/6N mice with 4 mg/kg rapamycin by intraperitoneal (i.p.) injection every other day for 6 weeks beginning at 20−22 months of age resulted in significant

* Tel: +1 206 543 4849, fax: +1 206 543 3644.
E-mail address: kaeber@uw.edu.

http://dx.doi.org/10.1016/j.jgg.2014.06.009

Table 1
Published studies showing lifespan extension from rapamycin in mice

Mouse strain	Rapamycin dosing/delivery	Rapamycin in blood (ng/mL)	Lifespan effect	Reference
UMHET3	14 ppm (~2.24 mg/kg/day) encapsulated in food beginning at 600 days	60–70	Maximum lifespan (90th percentile) increase of 9% and 14% for males and females, respectively.	Harrison et al., 2009
UMHET3	14 ppm encapsulated in food beginning at 9 months	NR	Mean lifespan increase of 10% and 18% for males and females, respectively. Maximum lifespan increase of 16% and 13% for males and females, respectively.	Miller et al., 2011
UMHET3	4.7 ppm encapsulated in food beginning at 9 months	7 (females); 6 (males)	Median lifespan increase of 3% and 16% for males and females, respectively. Maximum lifespan increase of 6% and 5% for males and females, respectively.	Miller et al., 2014
UMHET3	14 ppm encapsulated in food beginning at 9 months	16 (females); 9 (males)	Median lifespan increase of 13% and 21% for males and females, respectively. Maximum lifespan increase of 8% and 11% for males and females, respectively.	Miller et al., 2014
UMHET3	42 ppm encapsulated in food beginning at 9 months	80 (females); 23 (males)	Median lifespan increase of 23% and 26% for males and females, respectively. Maximum lifespan increase of 8% and 11% for males and females, respectively.	Miller et al., 2014
C57BL/6N	14 ppm encapsulated in food beginning at 19 months	3–4	Increased in females. No percentage given.	Zhang et al., 2014
C57BL/6N	4 mg/kg i.p. injection every other day for 6 weeks beginning at 20–22 months	NR	80% survival in treated group compared to 20% survival in controls at 30 months.	Chen et al., 2009
C57BL/6J	14 ppm encapsulated in food initiated at 4, 15, or 20–22 months	4.6	Increased median lifespan in males; no percentage given; survival study not completed.	Neff et al., 2013
129/Sv	1.5 mg/kg rapamycin three times per week for two weeks out of every month beginning at 2 months of age	NR	At 800 days 54% of rapamycin treated animals were alive compared to 36% of controls. At 900 days of age 31% rapamycin treated were alive and 10% of controls were alive.	Anisimov et al., 2011

NR = not reported.

improvements in hematopoietic stem cell function, as assessed by successful vaccination against influenza virus. Strikingly, a partial survival analysis reported in the same study showed that a similar rapamycin treatment regimen significantly enhanced survival at 30 months of age from around 20% for the control cohort to around 80% for the rapamycin treated cohort. Unfortunately, the full survival analysis was not reported, so it remains unclear what the actual magnitude of lifespan extension from this transient higher-dose rapamycin treatment might have been.

CHALLENGES WITH ATTEMPTING TO ASSESS WHETHER RAPAMYCIN "SLOWS AGEING" AT SUB-OPTIMAL DOSES

One major reason why it is critical that we obtain a better understanding of the dose response profile for rapamycin with respect to longevity is that it is difficult, if not impossible, to rigorously assess the full impact of rapamycin on healthspan using sub-optimal doses. To illustrate this point, we should consider two recent studies which aimed at assessing whether rapamycin slows ageing in mice by quantifying the effects of the 14 ppm rapamycin diet on age-related phenotypes (Wilkinson et al., 2012; Neff et al., 2013). Both studies detected improvements in some, but not all, of the age-sensitive parameters they measured in the rapamycin treated animals compared to untreated controls. Yet, the two studies reached opposite conclusions as evidenced by their titles: "Rapamycin slows ageing in mice" (Wilkinson et al., 2012) and "Rapamycin extends murine lifespan but has limited effects on ageing" (Neff et al., 2013). Clearly, both interpretations cannot be correct.

These studies, and others like them, are based on the idea that if rapamycin is extending lifespan by slowing ageing, then most age-related declines in function should also be delayed by rapamycin. While this is a logical assumption, it does not necessarily follow that all age-sensitive traits will show a similar magnitude of response to a given dose of rapamycin. In other words, just because median lifespan is increased by 10% when mice are fed a diet containing 14 ppm rapamycin, it need not be the case that all age-associated cancers, age-associated cardiac dysfunction, age-associated cognitive decline, etc. will each also be attenuated by 10%. Instead, it is almost certain that different age-associated phenotypes will have differential responses to any given "anti-ageing" intervention, and it is overly simplistic to think that a single, sub-optimal dose of rapamycin would yield detectable effects on all age-sensitive