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How longevity research can lead to therapies for Alzheimer's disease: The rapamycin story

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ABSTRACT

The discovery that rapamycin increases lifespan in mice and restores/delays many aging phenotypes has led to the speculation that rapamycin has 'anti-aging' properties. The major question discussed in this review is whether a manipulation that has anti-aging properties can alter the onset and/or progression of Alzheimer's disease, a disease in which age is the major risk factor. Rapamycin has been shown to prevent (and possibly restore in some cases) the deficit in memory observed in the mouse model of Alzheimer's disease (AD-Tg) as well as reduce Aβ and tau aggregation, restore cerebral blood flow and vascularization, and reduce microglia activation. All of these parameters are widely recognized as symptoms central to the development of AD. Furthermore, rapamycin has also been shown to improve memory and reduce anxiety and depression in several other mouse models that show cognitive deficits as well as in 'normal' mice. The current research shows the feasibility of using pharmacological agents that increase lifespan, such as those identified by the National Institute on Aging Intervention Testing Program, to treat Alzheimer's disease.

1. Introduction

Rapamycin (also known as Sirolimus) is macrocyclic lactone produced by the bacterium *Streptomyces hygroscopicus* isolated from soil samples from Easter Island. In 1975, Vezina et al. showed that cultures of *S. hygroscopicus* inhibited the growth of fungi with no activity against gram-positive and gram-negative bacteria and low toxicity in mice (Vezina et al., 1975). Based on the initial studies, rapamycin was developed as an antifungal agent; however, because it was found to have immunosuppressive properties, this line of research was discontinued. In 1988, it was discovered that rapamycin had antirejection properties without the side effects associated with other antirejection agents (Camardo, 2003). This discovery led eventually to FDA approving in 1999 the use of rapamycin in combination with other immunosuppressive agents to prevent the rejection of organs in transplant patients (Camardo, 2003).

A major breakthrough occurred in 1994, when three groups showed that rapamycin bound a specific protein, Target of Rapamycin (TOR) (Cafferkey et al., 1994; Brown et al., 1994; Sabatini et al., 1994). TOR was subsequently found to be the serine/threonine kinase that was the regulatory nexus in the response of eukaryote cells to nutrients, growth factors, and cellular energy status. In mammals, TOR (mTOR) forms two major complexes: mTORC1, which is inhibited by rapamycin (Caron et al., 2010) and mTORC2, which has been reported to be insensitive to rapamycin; however, recent data suggest that long term rapamycin treatment might inhibit mTORC2 (Sarbassov et al., 2006). The mTORC1 consists of mTOR, Raptor, mLST8, FKBP38, PRAS40, and Deptor, and through specific binding of rapamycin to FKBP12, rapamycin inhibits the activity of mTORC1 leading to a decrease in protein synthesis, increased autophagy and inhibition of cell growth (Stanfel et al., 2009).

2. Effect of rapamycin on longevity

The TOR signaling pathway is an attractive candidate to study with respect to aging because it has the potential to affect a large number of processes regulated by TOR signaling that could have a major effect on an organism. In the early 2000s, investigators began studying the effect of TOR on lifespan of invertebrates. Mutations in TOR increased the lifespan of yeast (Kaeberlein et al., 2005), *Caenorhabditis elegans* (Vellai et al., 2003; Jia and Levine, 2007; Hansen et al., 2007), and *Drosophila* (Kapahi et al., 2004). These data provided the first evidence that increased longevity could be achieved by reduced TOR signaling and suggested that rapamycin, which inhibits TOR signaling, might increase lifespan in other species, including mammals. In 2009, the National

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Institute on Aging Interventions Testing Program reported that feeding rapamycin to mice significantly increased lifespan, both mean and maximum (Harrison et al., 2009). Not only was this the first report to show that a pharmacological agent could increase lifespan of a mammal, the increase in lifespan was observed in both male and female mice on a heterozygous background: (BALB/cByJ × C57BL/6J) F1 females crossed to (C3H/HeJ × DBA/2J) F1 males, which is referred to as UM-HET3 mice. Thus, rapamycin's effect on lifespan is likely to apply to mice in general, i.e., it is not unique to a specific inbred strain of mice. In addition, the increase in lifespan was replicated at three independent sites, and the increase in lifespan was achieved when administered relatively late in life (e.g., 19 months of age, which would be similar to ~65 years of age in humans). This study was selected by *Science* as one of the major scientific breakthroughs in 2009 (*Science* 326, 1598–1607). The importance of showing that the increase in lifespan was increased when rapamycin was administered late in life cannot be understated because all previous experimental manipulations that increased the lifespan of mammals (mice or rats) were implemented early in life. Thus, the study by Harrison et al. (Harrison et al., 2009) provided the first proof of principle that a pharmacological agent could be developed to slow down aging in humans, i.e., an anti-aging pill was not a dream but theoretically possible.

Since the initial publication in 2009, 15 reports have been published on the effect of rapamycin on the lifespan of mice, and these studies are listed in Table 1. The large number of studies on the lifespan of mice in only five years is impressive and gives us an ability to generalize about how rapamycin affects longevity. The current studies show that rapamycin not only increases the lifespan of various strains of 'normal' laboratory mice, but also increases the lifespan of genetically modified mouse models of various human diseases. In studies in which male and female mice have been compared, rapamycin was found consistently to have a greater effect on the lifespan of female mice. Of the 16 studies in which the lifespan of rapamycin treated mice has been studied, only two studies, both with transgenic mouse models of ALS (amyotrophic lateral sclerosis), show no increase in lifespan. Thus, the effect of rapamycin on lifespan is very robust and reproducible in various strains and genetic models of mice. However, the studies with the ALS transgenic mice indicate that rapamycin's longevity effect may not be universal and that some genotypes do not respond to rapamycin. It is also possible that rapamycin might shorten the lifespan of some genetic models. A recent study by the National Institute on Aging Interventions Testing Program compared the effect of various doses of rapamycin on the lifespan of male and female UM-HET3 mice (Miller et al., 2014). Longevity was shown to be dose-dependent from one-third to 3-fold the concentration used initially by Harrison et al. (2009). Thus, the effect of rapamycin on the lifespan of mice occurs over a broad dose range.

Table 1
Effect of rapamycin on lifespan of mice.

Reference	Mouse strain	Age initiated	Increase in lifespan
Control/wild type mice			
Harrison et al. (2009)	UM-HET3	19 months	9% M &14% F
Miller et al. (2011)	UM-HET3	9 months	10% M &18% F
Anisimov et al. (2011)	129/Sv	2 months	10% F
Neff et al. (2013)	C57BL/6	4, 13, & 20 months	11% M
Miller et al. (2014)	UM-HET3	9 months	3–23% M & 16–26% F
Zhang et al. (2014)	C57BL/6	19 months	nc M & 6% F
Fok et al. (2014)	C57BL/6	4 months	11% M & 16% F
Genetically modified mice			
Fujishita et al. (2008)	Apc^{D716}	6–14 weeks	140–220% M/F
Anisimov et al. (2010)	HER-2	2 months	13% F
Comas et al. (2012)	$p53^{-/-}$	2 months	30% M
Ramos et al. (2012)	$Lmna^{-/-}$	3–4 weeks	23–57% M/F
Komarova et al. (2012)	$p53^{+/-}$	~5 months	10–28% M
Livi et al. (2013)	$Rb1^{+/-}$	8–10 week	14% M–9% F
Johnson et al. (2013)	$Ndufs4^{-/-}$	~20 days	25% M–38% F
Hasty et al. (2014)	$APC^{Min/+}$	50 days	280–440% F
Zhang et al. (2011)	G93A	64 days	nc Sex?
Bhattacharya et al. (2012)	H46R/H48Q	64 days	nc M/F

The percent increase in lifespan (nc = no change) is shown for male (M) and female (F) mice or a combination of male and female mice (M/F). The strain of mouse or the genetic mouse model is given with the age that the rapamycin treatment was initiated.

The observation that rapamycin increases lifespan (both mean and maximum) in mice strongly suggests that rapamycin increases lifespan by slowing down aging. In the past two years, several studies have examined the effect of rapamycin on various parameters of healthspan. Currently, it appears that while rapamycin improves some measures of physiological function that decline with age, other functions are not altered by rapamycin. However to date, no physiological function that changes with age is negatively altered by rapamycin (Richardson, 2013). One of the major consequences of aging, which leads to reduced quality of life and increased medical costs seen in the elderly, is the occurrence of a wide variety of pathological conditions. Because animal studies in which aging has been altered show a reduced/delayed incidence of most age-related diseases, it is possible that rapamycin might have a broad protection against age-related diseases in humans. The current limited data support this possibility. For example, it is clear that rapamycin has a dramatic effect on cancer in mice (Sharp and Richardson, 2011), most likely because of its anti-growth and anti-proliferation properties. In fact, it has even been argued that the life extension seen with rapamycin is due to its anti-cancer action rather than an anti-aging action (Neff et al., 2013). In addition, several studies have shown that rapamycin reduces atherosclerotic plaque formation in mouse models of atherosclerosis (Pakala et al., 2005; Mueller et al., 2008). Although rapamycin has been reported to improve various motor-tasks in models of Huntington disease (Ravikumar et al., 2004) and Parkinson's disease (Malagelada et al., 2010), there was no information on the effect of rapamycin on Alzheimer's disease before 2010 and very little information on the effect of rapamycin on cognition. In fact, the few early studies suggested that rapamycin might have a negative effect on memory, e.g., reduced long-term memory facilitation and consolidation (Casadio et al., 1999; Tischmeyer et al., 2003) and long-term plasticity in the brain (Tang et al., 2002). The focus of this report is to describe the pioneering studies in which the effect of rapamycin on Alzheimer's disease was tested in transgenic mice genetically manipulated to mimic Alzheimer's disease (AD-Tg mice). In addition, we review the current studies on the effect of rapamycin on cognition/memory in other mouse models with cognitive impairment as well as 'normal' laboratory mice.

3. Effect of rapamycin on Alzheimer's disease

3.1. Cognition/memory

The first studies to test the effect of rapamycin on memory in AD-Tg mice were initiated shortly after the 2009 *Nature* publication on lifespan and conducted independently by the laboratories of Oddo and Galvan. They found, as shown in Fig. 1, that rapamycin prevented the loss of memory/cognition in two different transgenic mouse models of AD (Caccamo et al., 2010; Spilman et al., 2010). The 3xTg-AD mice used by Oddo's laboratory harbors mutant forms of three human genes associated with AD: amyloid protein precursor (APP), tau, and presinilin-1. The 3xTg-AD mice are the only mouse model of AD that develops both Aβ-containing plaques and tau-containing tangles. The 3xTg-AD mice show significant memory deficits, which can be first detected around 6 to 8 months of age (Oddo et al., 2003). The hAPP(J20) used by Galvan's laboratory overexpresses a minigene of APP carrying the Swedish and Indiana FAD mutations. The hAPP(J20) mice show an increase in soluble Aβ levels in brain and synaptic deficits starting at 3 months of age, age-dependent AD-like decline in learning and memory that can be detected at seven months of age, and deposition of Aβ plaques starting at ~10 months of age that steadily increases with increasing age (Mucke