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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 A β 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.

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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 (Sarbasov 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 (Kaeblerlein 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.

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.

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.

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 presenilin-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

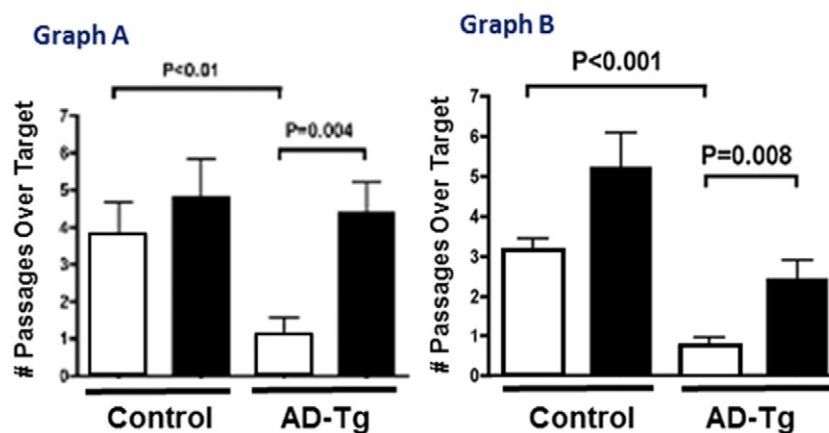


Fig. 1. Effect of Rapamycin on the Memory of AD-Tg Mice assessed by the Morris water maze. Graph A: data taken from Caccamo et al. (2010) for 3XTg-AD (AD-Tg) and non-transgenic (Control) mice fed rapamycin (black bars) or a control diet (white bars) starting at 6 months of age for 10 weeks. Graph B: data were taken from Spilman et al. (2010) for hAPP(J20) (AD-Tg) and non-transgenic (Control) mice fed rapamycin (black bars) or a control (white bars) diet starting at 4 months of age for 13 weeks.

et al., 2000; Hsia et al., 1999). The data in Fig. 1 shows that rapamycin administration to young/adult AD-Tg mice for 10 to 13 weeks prevented the loss of cognition observed in the two AD-Tg mouse models studied as measured by the Morris water maze. In fact, there was no significant difference between the control non-treated mice and the AD-Tg mice treated with rapamycin. The dose of the encapsulated rapamycin, which was delivered in the diet fed the AD-Tg mice, was the same as that used in the studies showing rapamycin increased lifespan in UM-HET3 (Harrison et al., 2009) and C57BL/6 mice (Fok et al., 2014). It should also be noted that rapamycin had no detrimental effect on memory/cognition in the control mice as suggested in early studies. In fact, under these experimental conditions, cognition in the control mice appeared to be slightly, but not significantly, improved.

While these studies show that rapamycin was able to prevent the initial loss of memory in the AD-Tg mice, it was of interest to determine whether rapamycin would block 'established' memory deficits in symptomatic animals that show cognitive deficits, a stage in the progression of AD-like disease that models mild cognitive impairment (MCI) in humans. Galvan's laboratory showed that feeding rapamycin for 16 weeks to 7-month-old hAPP(J20) mice, which show AD-like cognitive impairments, improved learning and restored memory to levels indistinguishable from those of wild-type controls (Lin et al., 2013). These data suggest that rapamycin may have the potential to treat cognitive impairment at the earliest stage of AD.

Oddo's laboratory studied the effect of rapamycin treatment over the lifespan of the AD-Tg mouse to determine if rapamycin could prevent loss of memory. They showed that treating 2-month-old 3XTg-AD mice with rapamycin for 16 months completely prevented the loss of memory; the rapamycin treated 3XTg-AG mice at 18-months of age performed as well as 18-month-old control mice (Majumder et al., 2011). However, giving the 3XTg-AD mice rapamycin for 3 months starting at 15 months of age, an age at which mice show widespread plaques and tangles and profound memory deficits, had no effect on memory indicating that rapamycin might have little effect on cognition if given at later stages of AD. Interestingly, Galvan's laboratory recently found that rapamycin treatment in young/adults could protect AD-Tg mice from cognitive deficits later in life. They found that hAPP(J20) mice fed rapamycin for 16 weeks starting at 7 months of age, not only improved cognition immediately after feeding rapamycin (Lin et al., 2013) but was also sufficient to maintain cognitive function late in life, such that 28-month-old hAPP(J20) mice given rapamycin for 16 weeks at 7 months of age showed no detectable cognitive deficits in a spatial novelty task (Veronica Galvan personal communication). Therefore, the current data with AD-Tg mice show that the loss of memory can be prevented by rapamycin and that rapamycin-treatment can restore memory if implemented shortly after the onset of memory impairment; however,

rapamycin may have little effect when given at late stages of the disease after overt plaque and tangle deposition.

3.2. Plaques, tangles, and autophagy

One of the hallmark features that characterize AD is the accumulation of A β -containing plaques and tau-containing tangles, which are believed to play a major role in the decline in memory. As shown in Fig. 2(A–F), Oddo's laboratory found that rapamycin treatment reduced the accumulation of A β levels and fibrillar aggregates of A β approximately 40 to 50% in the 3XAD-Tg mice fed rapamycin for 16 months (Majumder et al., 2011). In addition, rapamycin reduced tau-aggregation as measured by tau phosphorylation (Fig. 2G, H). Microglia activation, which is an invariable feature of AD pathology, was also observed to be dramatically reduced in the rapamycin treated AD-Tg mice (Fig. 2I, J). Consistent with these observations, Galvan's laboratory has also documented that rapamycin reduced levels of A β in the brains of hAPP(J20) mice after a few months (Spilman et al., 2010) and amyloid plaques at later stages of the AD-like disease (Lin et al., 2013).

Because rapamycin is known to induce autophagy and because autophagy is the primary pathway cells use to degrade protein aggregates, both Galvan's and Oddo's laboratories studied the role of autophagy in the reduction of plaques and tangles in the brains of AD-Tg mice. They showed that markers of autophagy were induced in the brains of hAPP(J20) mice (Spilman et al., 2010) and 3XTg-AD mice (Caccamo et al., 2010). In addition, Oddo's laboratory showed that rapamycin treated mice show a greater localization of A β in lysosomes (60% vs 20%), suggesting a more active degradation of these peptides after rapamycin treatment. Oddo's laboratory went on to show that the decrease in A β levels induced by rapamycin in cells expressing A β could be prevented by blocking autophagy. More recently, Oddo's laboratory showed that rapamycin administration decreased tau pathology in a mouse model of tauopathies, indicating that rapamycin might be beneficial not only for AD but also for other tauopathies (Caccamo et al., 2013). Therefore, it appears that the induction of autophagy by rapamycin plays a major role in the decrease in the accumulation of plaques and tangles in the brains of the AD-Tg mice and possibly the improvement in memory.

Oddo's laboratory also has taken a genetic approach to determine if the effect of rapamycin on cognition and plaque accumulation occurred through mTOR. Although rapamycin is an mTOR inhibitor, there is growing evidence that it may also have effects independent of mTOR (Malagelada et al., 2010; Thoreen and Sabatini, 2009). For example, rapamycin suppresses mTOR-dependent translation of some classes of mRNAs but not others (Choo et al., 2008). In addition, rapamycin binds to L-type voltage-dependent Ca²⁺ channels, and it is thought that this binding may mediate some of the neuroprotective properties

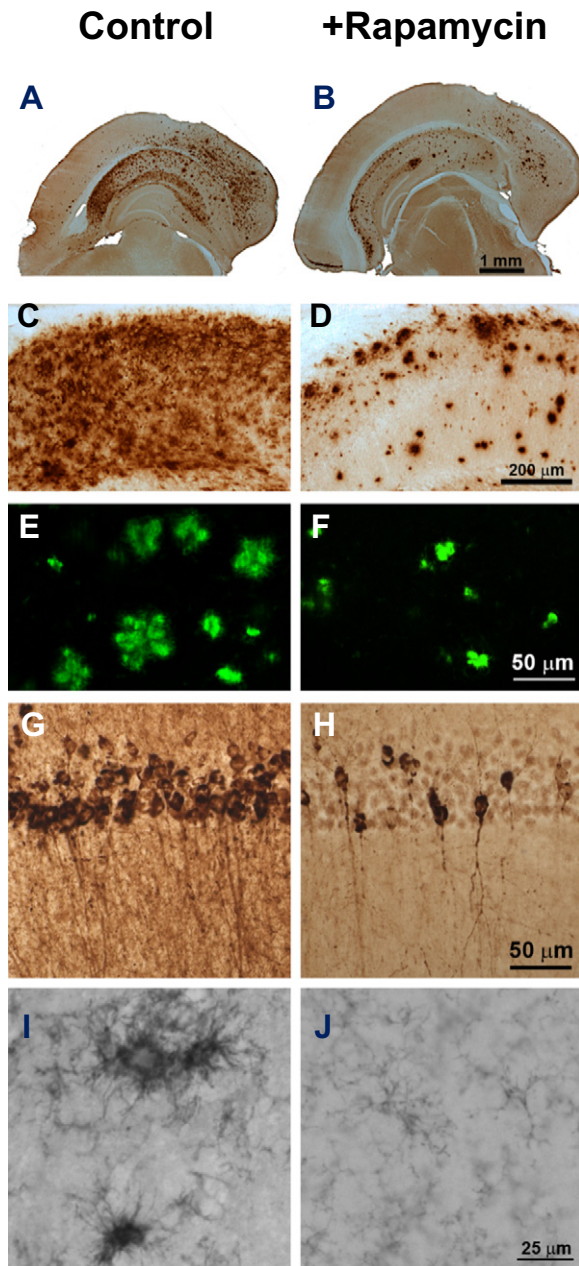


Fig. 2. Effect of rapamycin on plaques and tangles in brains of 3XTg-AD mice. The levels of A β_{42} in whole brain (A, B) or hippocampus (C, D), fibrillar aggregates of A β (E, F), tau pathology as measured by hyperphosphorylation at Ser212 and Thr214 (G, H), and microglia activation (I, J) were measured in 2-month-old mice fed rapamycin or a control diet for 16 months.

Data taken from Majumder et al. (2011).

of rapamycin (Ruan et al., 2008). Oddo's laboratory has selectively removed one copy of the mTOR gene in the brains of the Tg2576 mice, a widely used animal model of AD. They found that genetic suppression of mTOR signaling reduced A β deposits and rescued memory deficits (Caccamo et al., 2014), mimicking the rapamycin-mediated improvements. These data strongly suggest that the rapamycin effects on AD-like pathology in mice are mediated through mTOR signaling.

3.3. Cerebral blood flow (CBF) and vascular function

In addition to the accumulation of plaques and tangles, vascular dysfunction is a phenotype of AD (Nagata et al., 2000). Using small animal imaging, Galvan's laboratory in collaboration with Ai-Ling Lin employed

imaging techniques similar to those used in humans to determine the effect of rapamycin on vascular function (Lin et al., 2013). As shown in Fig. 3(A, B), they found that CBF was restored to wild type levels when the hAPP(J20) mice were treated with rapamycin for 16 weeks. The restoration of CBF in hAPP(J20) mice was not due to changes in glucose metabolism but appeared to arise from changes in vascular density as shown in Fig. 3(C, D); rapamycin restored vascular density in the brains of hAPP(J20) mice to the levels found in the brains of control, untreated hAPP(J20). Ai-Ling Lin has also found that rapamycin improves CBF in old rats, LDL-receptor mice fed a high-fat diet, and APOE4 transgenic mice (personal communication); thus, rapamycin's ability to restore vascular function in the brain is not limited to AD.

The data obtained by Lin et al. (2013) also indicated that rapamycin acts acutely as an NO-dependent vasodilator, as had been suggested by Cheng et al. (2008). Therefore, they propose that rapamycin's activation of nitric oxide synthase is critical for the restoration of CBF in hAPP(J20) mice. Because the deposition of A β in the brain vasculature has been shown to cause microvessel disruption (Zlokovic, 2011), it is possible that the improvement in vascularization in rapamycin-treated AD-Tg mice is accompanied by decreased A β deposition within brain vasculature. Lin et al. (Lin et al., 2013) measured the amount of A β associated with brain blood vessels in rapamycin-treated and untreated hAPP(J20) mice. Fig. 3(E, F) shows that the A β associated with brain was significantly reduced in rapamycin-treated hAPP(J20) mice, which was correlated to reduced microhemorrhages (see Fig. 3M, N in (Lin et al., 2013)).

In summary, data generated over the past four years show that rapamycin can prevent, and possibly reverse, the complete AD phenotype observed in transgenic mouse models of AD, e.g., memory is improved, the accumulation of A β - and tau-aggregates is reduced, microglia activation is prevented, and CBF and brain vascular density are restored. Based on the current data, we propose that rapamycin prevents AD by acting on several pathways that can potentially lead to cognitive impairment as shown in Fig. 4. First, rapamycin increases the clearance of A β by restoring vascular integrity and CBF, and second, rapamycin increases the degradation of A β and tau aggregates by inducing autophagy, thereby counteracting the hyperactivation of mTOR that is observed in the brains of AD-Tg mice and patients (Oddo, 2012; Sun et al., 2014).

4. Effect of rapamycin on mouse models that are cognitively impaired

Studies over the past 5 years have shown that rapamycin can prevent cognitive impairment that occurs in a number of disorders/conditions that does not involve the accumulation of plaques/tangles. One of the first studies showing the benefits of rapamycin treatment on cognition was reported for mice heterozygous for *Tsc2* (tuberous sclerosis protein 2). *Tsc1* and *Tsc2* form a protein-complex that has tumor suppressor activity and also inhibits mTOR activity. Mutations in these two genes result in hyperactivation of mTOR that is characterized by hamartomas, benign growth of tumors in various organs. However, mutations in these genes also produce changes in the central nervous system separate from tumor growth. Ehninger et al. (2008) showed that *Tsc2*^{+/-} mice have deficits in learning and memory in the absence of neuropathology and seizures, and rapamycin treatment of these mice rescued the behavioral deficits in the *Tsc2*^{+/-} mice, including the impairment in memory. Since this report, rapamycin has been shown to prevent/restore the loss of memory arising from a number of other disorders/manipulations, which are associated with the hyperactivation of mTOR, and these studies are listed in Table 2. These disorders/conditions range from drug/alcohol treatment to induce seizures, to genetic manipulations that result in autism-like phenotypes. In addition, Erlich et al. (2007) reported that treating mice with rapamycin after brain trauma enhances the recovery of the brain from injury. Thus, the current literature shows that rapamycin has a broad range of effects on the central nervous system. This should not be surprising because components of the mTOR pathway are widely expressed in the central nervous system, especially *Tsc1* and 2, which

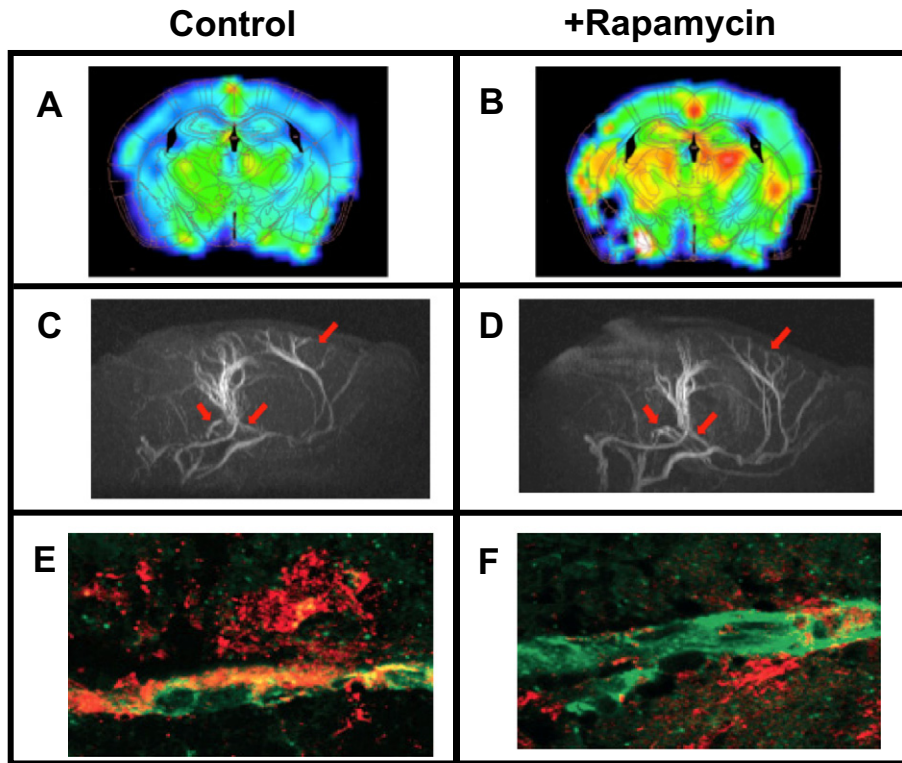


Fig. 3. Effect of rapamycin on CBF, vascularization, and cerebral amyloid angiopathy in hAPP(J20) mice. Panels A and B show CBF in various brain regions with brighter colors indicating increased blood flow. Panels C and D show magnetic resonance angiography images of brains showing vasculature with the arrows pointing to regions of the brain where rapamycin restored vascular density. Panels E and F show A β (shown in red) associated with brain blood vessels (shown in green). The data were taken from Lin et al. (2013) for 7-month-old mice fed rapamycin or a control diet for 16 weeks.

are highly abundant in the adult central nervous system and play a major role in regulating mTOR activity (Ehninger and Silva, 2011).

5. Effect of rapamycin on cognition and behavior of ‘normal’ laboratory mice

The first study to show that rapamycin increased cognition of ‘normal’ laboratory mice was conducted by Oddo’s laboratory in 2012 (Majumder et al., 2012). They found that 18-month-old mice treated with rapamycin for 16 months showed significantly improved spatial memory on the Morris water maze compared to the mice fed a control diet. Subsequently, Galvan’s laboratory (Halloran et al., 2012) and Neff

et al. (Neff et al., 2013) also showed that rapamycin improved spatial memory of mice on the Morris water maze. Fig. 5 compares the Morris water maze data generated by these three groups. In addition, Galvan’s group showed that rapamycin restored the memory of old mice on a passive avoidance task. Majumder et al. (2012) found that the improvement in cognition observed with rapamycin was associated with decreased brain levels of the pro-inflammatory cytokine, IL-1 β , and increased NMDA signaling; both have been shown to affect brain function. It should be noted that Erlich et al. (Erlich et al., 2007) found that rapamycin reduced inflammation as shown by decreased numbers of activated microgila in their brain injury model, and several studies suggest that rapamycin reduces inflammation in various tissues (Chen

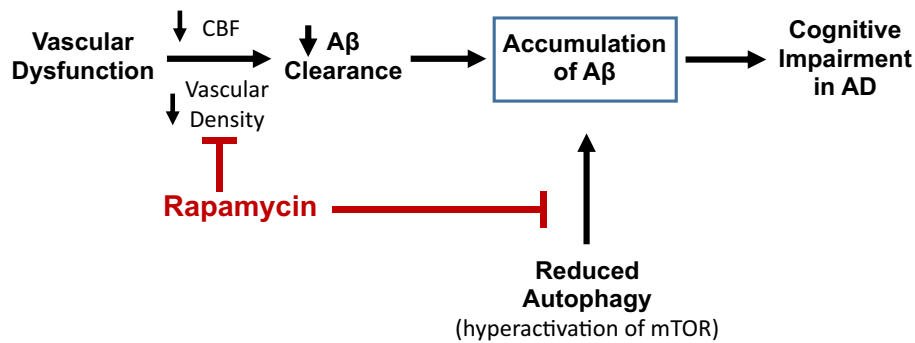


Fig. 4. Diagram showing the protective effect of rapamycin in AD using the ‘two-hit’ process described by Zlokovic (2011). Rapamycin prevents the ‘first hit’ by increasing the clearance of A β through the restoration of vascular integrity. Rapamycin also blocks the second ‘hit’ by inducing autophagy preventing the accumulation of A β in the brain counteracting the hyperactivation of mTOR, which occurs in the brains of AD-Tg mice and patients.

Table 2
List of neurological disorders improved by rapamycin treatment.

Disorder/condition	Rapamycin treatment	Effect of rapamycin	Reference
Head injury <i>Tsc2</i> ^{+/-} Mice	4 h after head trauma Treated 3 h to 5 days continuously before assays.	Greater improvement of markers of neurobehavioral function Corrects deficits in memory/learning (Morris water maze and the 8-arm radial maze) and contextual discrimination.	Erlich et al. (2007) Ehninger et al. (2008)
THC-cannabinoid treatment	Pre-treatment for 5 days	Prevented decline in performance on the novel object recognition test	Puighermanal et al. (2009)
Alcohol addiction	1 dose	Attenuates conditioned preference to alcohol, e.g., decreased binge drinking and motivation to consume alcohol	Neasta et al. (2010)
Deletion of <i>Tsc1</i> in the cerebellar Purkinje cells	Started at P7	Prevented deficits in cognition on the water T-maze, pathological phenotypes of autism in the brain, and autistic-like behavior	Tsai et al. (2012)
Pilocarpine-induced seizures	Post-treatment every other day for 8 days	Restoration of cognition as measured on the Morris water maze and the novel object recognition test	Brewster et al. (2013)
<i>Disc1</i> knockdown in dentate granule neurons	Treatment 5–13 days after retroviral injection	Reversed deficits in cognition (object-place recognition task) as well anxiety phenotype (elevated plus maze)	Zhou et al. (2013)

et al., 2009; Bonegio et al., 2005; Dubois et al., 2003). Therefore, some of the benefits arising from rapamycin treatment might occur through rapamycin's anti-inflammatory actions.

In addition to studying the effect of rapamycin on memory, Galvan's laboratory studied the effect of rapamycin on non-cognitive components of behavior, specifically anxiety- and depressive-like behaviors (Halloran et al., 2012). They found that 4-month-old mice treated with rapamycin for 16 weeks showed reduced anxiety-like behavior, e.g., reduced thigmotaxis (swimming in close proximity to the pool wall) and reduced aversion to open spaces using an elevated plus maze. Rapamycin also was found to reduce depressive-like behavior, e.g., floating during training phase of the Morris water maze and reduced time spent immobile on the tail suspension test. Similar effects of rapamycin on behavior have been reported for genetically modified mice that show autistic-like behavior. Mice with mutations in *Tsc2* or *Disc1* genes, show increased anxiety, hyperexcitability, abnormal social interaction, repetitive behavior and vocalizations, all of which are attenuated by rapamycin treatment (Tsai et al., 2012; Zhou et al., 2013). Recently, Kolosova et al. (2013) reported that rapamycin treatment decreased anxiety and improved locomotor and exploratory behavior in senescence-accelerated OXYS rats. In addition, Neasta et al. (2010) showed that rapamycin attenuates the conditioned preference of rats and mice to alcohol. Therefore, rapamycin affects a wide variety of cognitive and non-cognitive types of behavior in rodents. Galvan's laboratory showed that the changes in anxiety- and depressive-like behavior were correlated with increased levels of dopamine and dopamine metabolites in the midbrain.

6. Conclusion

Research over the past four years has shown that rapamycin can have quite profound effects on the central nervous system of mice, resulting in

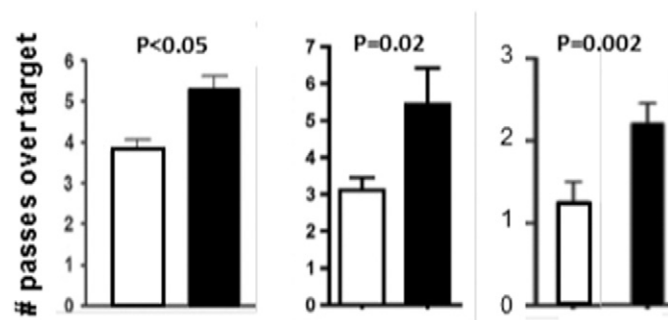


Fig. 5. Effect of rapamycin on memory of 'normal' laboratory mice. Mice were fed rapamycin (black bars) or a control diet (white bars) for various lengths of time and cognitive performance measured by the Morris water maze. The left graph is 2-month-old mice fed rapamycin for 16 months (data taken from Majumder et al., 2012). The middle graph is 4-month-old mice fed rapamycin for 16 weeks (data taken from Halloran et al. (2012)). The right graph is 4-month-old mice fed rapamycin for 11 months. Data taken from Neff et al., 2013).

both improved memory and changes in behavior, e.g., anxiety and depression. As shown in Fig. 6, the changes in behavior and cognition observed in mice could arise through a number of pathways that have been reported to be altered by rapamycin. Many of these pathways could also play an important role in preventing the progression of Alzheimer's disease.

The studies from Oddo's and Galvan's laboratories show quite convincingly that rapamycin given to mice at a dose shown to increase lifespan and delay many aging phenotypes and pathologies prevented, and may have restored, the loss in cognition observed in two different transgenic mouse models of Alzheimer's disease. As noted in a recent review, despite over a thousand clinical trials related to AD, only five drugs are currently approved for treatment of the disease (Cavanaugh et al., 2014); unfortunately, these five drugs have no effect on the progression of AD. Also disappointing has been the inability to translate to humans therapies that prevent deficits in cognition in AD-Tg mouse models. It has been argued that the lack of translation from mice to humans is due to species differences in brain structure and complexity. It is also possible that the types of mouse models used do not accurately mimic sporadic AD, which accounts for over 95% of the cases of AD. For example, all the AD transgenic mouse models overexpress in the brain various human genes containing mutations associated with familial AD. These AD-Tg mice show a loss of cognition and accumulation of A β aggregates early in life, comparable to ~20-year-old human. Therefore, the AD-Tg mice currently used to study AD may not be good

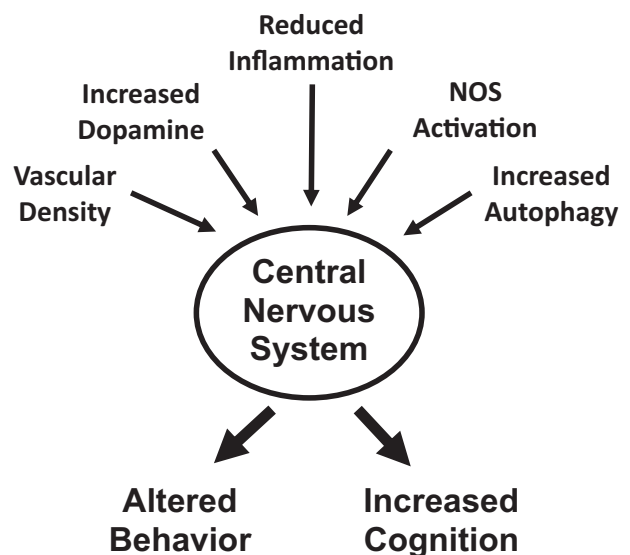


Fig. 6. Diagram showing pathways reported to change with rapamycin treatment that could impact the changes observed in behavior and cognition observed in laboratory rodents.

models of sporadic AD, which occurs late in life (after 65 years of age). In addition, most of the recent therapies developed to treat AD have focused on reducing the accumulation of A β aggregates, either by preventing the processing of A β using inhibitors of γ - or β -secretases or using immunization strategies to remove A β deposits. While plaques and tangles are correlatively linked to disease symptoms of AD, they may not be causal or key in the mechanism initiating the disease.

We propose that rapamycin has greater likelihood of successfully treating AD than previous therapies studied for three reasons. First, rapamycin appears to have a major effect on aging in rodents, and age is the single greatest risk factor for AD. In fact, the initial study showed that rapamycin was effective in increasing lifespan when implemented late in life (Harrison et al., 2009). Therefore, altering pathways fundamental to aging would be a novel strategy for treating AD, and this strategy is possible now that we have pharmacological intervention into aging with rapamycin that affects a large number of age-related diseases and pathologies in mice (Richardson, 2013). Second, rapamycin appears to have a very broad effect on the central nervous system. It not only reduces the accumulation of both A β and tau-containing aggregates, it also improves and restores vascular function and CBF as well as reducing inflammation. Third, rapamycin has an effect on both cognition and behavior in mouse models other than AD-Tg mice, i.e., rapamycin appears to have a very broad effect on the central nervous system. Thus, we predict that rapamycin will potentially be successful in treating sporadic AD patients because of rapamycin's anti-aging properties, which affect a wide range of pathways (not just A β aggregation) that might be involved in the initiation and/or progression of sporadic AD, e.g., such pathways as proteostasis, inflammation, vascular dysfunction, etc.

One major advantage to using rapamycin (or its derivatives, called rapalogues) to treat AD is that it has been approved by the FDA since 1999 for various uses in humans. Therefore, the toxicity profiles of rapamycin and its rapalogues are well characterized (Soefje et al., 2011). The use of rapamycin and rapalogues in treatment of AD is also attractive because these compounds have been given to cancer patients for relatively long periods of time with little change in the quality of life (Mita and Mita, 2011). In fact, a pilot study by Lang et al. (Lang et al., 2009) reported that Everolimus, a derivative of rapamycin, affected cognition and depression in humans in a way that is similar to what has been observed in mice. In the study, nine cardiac transplant patients (66 \pm 6 years of age) who had initially received a standard immunosuppression regimen of calcineurin inhibitors were followed when they were switched to Everolimus. Four weeks after Everolimus treatment, significant improvements were observed in memory (Wechsler Memory Scale-Revised), depression (Beck Depression Inventory), and executive function (Trail Making Tests A and B).

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