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Preserving Youth: Does Rapamycin Deliver?

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Abstract

Research suggests that the drug rapamycin slows mammalian aging, but a provocative new study has gained attention by claiming to show it does not.

Scientists seeking strategies for slowing human aging were energized when the clinically approved drug rapamycin was shown to reproducibly extend lifespan and healthspan in a range of model systems, including yeast, nematodes, fruit flies, and mice (1). In particular, observations in mice that the drug both extends life and delays the onset or progression of multiple age-related phenotypes (2–6) have led scientists to speculate that rapamycin might be a starting point for the development of therapeutic agents that stem age-related diseases (7).

Rapamycin and its derivatives (rapalogues) are specific inhibitors of the mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase that regulates cell growth and proliferation. Rapalogues are used clinically to prevent organ-transplant rejection and restenosis associated with cardiac stents and in the treatment of some forms of cancer. The hypothesis that mTOR inhibition by rapamycin slows at least a subset of the molecular processes that drive cellular, tissue, and organismal aging was challenged recently by a report that describes experimental findings in mice treated with rapamycin for a one-year period (8). Although life span was extended, the authors detected a reversal of aging-related changes in fewer than half of the assays performed. On the basis of these negative findings, Neff *et al*. conclude that their results dissociate rapamycin's effect on longevity from its potential effects on processes associated with aging, and suggest that rapamycin increases longevity in mice only by reducing cancer. Here we critically evaluate this conclusion, both in terms of the specific data presented in the new report and in light of the greater body of literature on the effects of rapamycin on the aging process and age-related diseases.

LIFE SPAN VERSUS HEALTHSPAN

Neff *et al*. (8) set out to test the hypothesis that rapamycin slows aging in mice by examining the effect of dietary rapamycin on life span (longevity) and healthspan (the length of life spent free from severe age-related disease) in male C57Bl/6 mice. As shown previously, the authors found that rapamycin increased median life span by ~10% in male

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mice (females were not studied and maximum life span was not reported). However, improvements from rapamycin treatment were detected in only ~38% (15 of 40) of the healthspan measures quantified. The beneficially impacted healthspan measures—many of which had not been previously reported for rapamycin—included cardiac function, cancer incidence, some measures of cognitive function, tissue pathology, immune system preservation, renal and hepatic function, and muscular and visual performance. Muscle strength, vision, metabolic rate (measured by indirect calorimetry), steroid metabolism, DNA-damage accumulation in lung, and a variety of other histopathological phenotypes did not show improvement with rapamycin during aging in this study. Despite the various beneficial effects, Neff *et al*. conclude that rapamycin does not slow the aging process in general because of the number of healthspan measures that were not improved and because, in some cases where an improvement was observed, a similar change was also observed in younger animals after shorter-term rapamycin treatment.

Although Neff *et al.* should be commended for their examining a much larger number of healthspan parameters than has previously been attempted in any one study, their report suffers from limitations that make it difficult to interpret the negative results. First, the observation that an intervention enhances physiological function in a young-adult animal does not negate a role for that intervention in the modulation of intrinsic processes of biological aging. Previous studies on mechanisms of aging have provided no justification for this viewpoint. In fact, there is strong evidence that the opposite is true. For example, caloric restriction alters many physiological parameters in a variety of model organisms regardless of age and also extends life span and is widely considered to slow the aging process. As a second example, Neff *et al*. show a nearly complete reversal of age-related heart dimension and weight changes with rapamycin treatment but conclude that, because treatment was also associated with a reduction in heart weight in younger mice, the mechanism behind the rapamycin effect must be aging-independent. However, rapamycin had much greater effects on left-ventricular diameter and heart weight in the older animals, a finding reproduced by a recent study showing that 12 weeks of rapamycin treatment dramatically improves cardiac function in aged animals (9). Thus, we conclude that the cardiac findings are consistent with an attenuation of cardiac aging. Furthermore, we hypothesize that interventions which attenuate physiological effects of aging in older animals often will have a more modest beneficial effect in young-adult animals and that this may be particularly true when the intervention affects a progressive genetic, epigenetic, or metabolic effect of age.

The interpretation by Neff *et al.* that rapamycin promotes longevity solely through an anticancer mechanism also warrants careful examination. Neff *et al*. detect a significant reduction in cancer in 16-month-old mice treated with rapamycin from 4 to 16 months of age, but not in 22- to 25- month-old or 32- to 34-month-old animals treated with the drug from 13 to 25 months of age or ~22 to ~ 34 months of age, respectively. These results are consistent with the U.S. National Institute on Aging (NIA) Interventions Testing Program (ITP) studies, which have not detected large changes in tumor burden or frequencies of various tumor types in long-lived, rapamycin-treated UMHET3 mice. Thus, both studies find that late-life treatment extends life span without an apparent effect on cancer burden. Although Neff *et al*. interpret their results as evidence that rapamycin increases life span by

reducing cancer in mice only when treatment is initiated prior to 16 months of age, an alternative interpretation is that rapamycin delays cancer progression such that it scales with the increase in life span—as would be expected if the drug alters aging rate. Distinguishing between these interpretations requires an analysis of tumor size and burden and a determination of whether or not neoplasia was the cause of death. Because Neff *et al.* did not report such results, it is not possible to determine whether cancer reduction underlies the observed increase in life span.

Furthermore, the hypothesis that rapamycin extends life span in mice by acting primarily as an anticancer agent does not take into account previous findings that rapamycin treatment is sufficient to extend life span in yeast, worms, and fruit flies— species that don't normally get cancer. In these organisms, several lines of evidence suggest that mTOR inhibition extends life span through de-repression of autophagy, altered regulation of mRNA translation, and enhanced mitochondrial function (7). Because mTOR regulates all of these highly conserved processes in mammals in a manner similar to that in lower organisms, it seems likely that mTOR's longevity-control mechanisms would also be conserved.

Another limitation of the study by Neff *et al*., as well as the literature on rapamycin in mammalian aging in general, is the lack of a dose-response profile. Neff *et al*. used the same dietary delivery method and dose of rapamycin used by the ITP; although this regimen is sufficient to increase life span, other doses of rapamycin might yield more robust or different effects on life span and healthspan parameters. Delivery and uptake of rapamycin likely occurs in an a organ- and tissue-specific manner, with some organs and tissues achieving relatively more or less inhibition of mTOR signaling than others for a given dietary dose of rapamycin provided. Likewise, it also seems probable that effects on aging-related parameters will be organ- and tissue-specific for a given level of mTOR inhibition. Indeed, a recent study by Wu *et al.* (10) suggests precisely this. Using a hypomorphic mTOR mouse in which mTOR expression is reduced by ~75%, the authors observed a 20% increase in life span and functional preservation in many, but not all, tissues and organs.

It is unfortunate that only male mice were studied by Neff *et al*., because at the specified dose of rapamycin male mice show a less robust longevity response to mTOR inhibition than do female mice (1). We suggest that, for a study confined to one gender, it might have been more informative to choose the gender that is affected to a greater extent by the intervention, especially if the study is limited to only one dose. It is more difficult to convincingly demonstrate a negative result than a positive one, and testing of the gender that shows the weaker effect coupled with the low replicate number in most of the assays limits the ability to detect statistically significant differences that may be present. In addition, high inter-individual variability limits the statistical power of many murine assays, and standard statistical analyses are necessary to interpret the significance of a negative result. Because these analyses were not presented in Neff *et al.*, the reported negative results might stem from low assay sensitivity, low replicate number, or both. This latter scenario is supported by the supplemental data, particularly the cardiac, immunological, and hematological assays, in which the rapamycin-treatment groups showed a trend toward an attenuation of the age-related change in a majority of the assays. If these assays were underpowered, that might radically alter the conclusions of the manuscript.

Finally, longitudinal measurement of physiological function would have provided paired measurements at the onset of the experiment and after treatment of each animal; this protocol confers greater sensitivity for detecting intervention-related changes given the high inter-animal variability associated with many of the assays. Longitudinal assays for age-related parameters are routinely reported in studies of mouse aging, including echocardiography, calorimetry, and behavioral and serum-based assays, some of which were used by Neff *et al.* in a cross-sectional manner.

The study by Neff *et al*. study provides an important addition to the body of literature linking mTOR inhibition to improvements in longevity and healthspan in mice. However, in light of the noted deficiencies, the negative results should be interpreted cautiously. Given the large body of evidence that genetic or pharmacological inhibition of mTOR extends life span and delays age-related changes in yeast, worms, flies, and mice, we suggest that the new work supports the model that rapamycin promotes longevity by targeting some, but not all, core molecular processes that drive cellular and systemic aging.

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Table 1

Rapamycin attenuation of age-related pathologies in model systems

Data from studies in yeast, invertebrates (worms, flies), and various mouse strains mice, as well as from a few human clinical trials suggest that, in addition to extending life span, rapamycin can positively impact multiple age-related pathologies, as would be predicted for a direct modulator of aging rates.

Age-related disorder	Model system
Cardiac dysfunction	Invertebrate (*Drosophila melanogaster*), Murine (multiple mouse strains)
Macular degeneration	Murine, Human clinical trial
Cancer	Murine, Human clinical trial
Stem cell decline	Invertebrate, Murine
Cognitive decline	Murine
Neurodegeneration	Invertebrate (*Drosophila melanogaster*, *Caenorhabditis elegans*), Murine
Immune decline	Murine
Renal dysfunction	Murine