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Aging is considered a major risk factor and plays a critical role in the pathogenesis of a wide variety of diseases including cancer, Alzheimer's disease, diabetes, and cardiovascular diseases among others. Aging is an inevitable process, and (in mammals especially) is thought to be underpinned by nine hallmarks of aging including1:

1. Genomic instability
2. Telomere attrition
3. Epigenetic alteration
4. Loss of proteostatis
5. Disregulation of nutrient sensing
6. Mitochondrial dysfunction
7. Cellular senescence
8. Stem cell exhaustion
9. Alteration of intercellular communication

Broadly these hallmarks can be categorized as being on the systematic level (nutritional dysregulation), cellular level (cellular senescence, stem cell exhaustion, altered intercellular communication), and the molecular level (genomic instability, telomere shortening, epigenetic alteration, loss of proteostatis, compromised autophagy, and mitochondrial dysfunction).2 Some of these major hallmarks of aging have come under evaluation as potential targets for pharmaceuticals as a way to prevent or treat age related diseases.

The Role of Metformin

Recently metformin, a drug typically utilized for the management of blood glucose control in patients with type II diabetes, has come under evaluation as a potential pharmacological agent for the attenuation of aging. Epidemiological studies evaluating metformin have found reduction in all-cause mortality in both diabetic and non-diabetic patients. Promising studies in mice have found metformin to extend lifespan and improve health over this duration as well.3 The mechanism of action of metformin's impact on aging may be multimodal. Metformin is thought to help with deregulated nutrient-sensing in part by decreasing insulin and IGF-1 levels. It is also thought to have an impact on intracellular communication via activation of 5 adenosine monophosphate activated protein kinase (AMPK) and via this mechanism, reduction of expression of inflammatory cytokines. This same pathway may contribute to activation of telomeric repeat-containing RNA (TERRA) which is thought to reduce telomere shortening. Metformin may also support genetic stability via reduction of oxidative stress and reduction of DNA damage, though, more information is needed on this particular proposed mechanism of action.3 These are just a few of the mechanisms by which metformin may play a roll in mitigation of pathogenic aging and via these mechanisms it has been proposed that metformin may work to mitigate a variety of age related diseases from degenerative skeletal diseases to cardiovascular diseases to neurogenerative diseases among others. One prospective study evaluated 86 normal glucose patients, 86 subjects with prediabetes treated with metformin, and 86 subjects with prediabetes not being treated with metformin over a period of 24 months. The study found that metformin therapy may help to reduce the risk of high-risk cardiovascular events (cardiac death, myocardial infarction, heart failure) by reducing coronary epithelial dysfunction as compared to the non-treatment prediabetes group, though, major cardiac events were higher for both prediabetes groups as compared to the normal glucose group.4 Other cohort or prospective studies evaluating patients with type II diabetes treated with metformin found decreased incidence of certain cancers such as colorectal cancer or breast cancer.5,6 Further randomized controlled trial level studies of metformin in patients with and without diabetes type II or prediabetes are needed to determine the role and extent of metformin in mitigation of common age related diseases, but limited information thus far is promising, making metformin an attractive candidate for future study.

The Role of mTOR Inhibitors

Another pharmacologic agent garnering interest for increasing healthy life expectancy is sirolimus (rapamycin). Sirolimus is an mTOR (mammalian target of rapamycin) inhibitor, mTOR is a protein kinase that is thought to play a role in several key hallmarks of aging including loss of protostasis, deregulated nutrient sensing, cellular senescence, mitochondrial dysfunction, and stem cell exhaustion. With regards to proteostatis, mTOR inhibition is expected to lead to a decrease in proteosynthesis which may in turn slow down aging via reduction of oxidative and proteotoxin stress. In terms of cellular senescence, mTOR may promote activity of senescence-associated secretory phenotype SASP), the activation of SASP has been shown to contribute to the secretion of proinflammatory mediators.7,8 Mitochondrial dysfunction has been shown to be associated with aging and age-related diseases, mTOR inhibition can assist with the clearing of old and dysfunctional mitochondria. Lastly, some studies in mice have found that sirolimus can restore the self-renewal capacity of some hematopoietic stem cells, thereby mitigating stem cell exhaustion.8

Studies are currently underway to investigate the potential of sirolimus as an mTOR inhibitor and an agent for mitigation of age-related disease. Studied doses of sirolimus vary widely in studies from just 2mg up to 12mg orally per day. Low dose sirolimus has also been studied topically for aging skin, and even intravitreally for macular degeneration, though, benefit was not noted in the case of the study for macular degeneration.9 One study in mice on sirolimus given orally beginning late in life found the treatment to extend the life of female and male rodents. The study used average age at 90% mortality of the population as a marker in the mice. In this study sirolimus fed female mice were on average 14% older, and male mice were on average 9% older at the 90% mortality time marker.10 Other studies expanding on this, and similar promising data looked to identify ideal dose and duration of sirolimus treatment for prolonging healthy life. One study evaluated 8mg/kg/day

injected intraperitoneally or delivered in their food at 126ppm for a period of 3 months, then evaluated lifespan in male and female mice. In the injection group, dramatically increased lifespan was noted in male mice, whereas adverse effects in female mice were noted and a lifespan benefit was not. For the group fed 126ppm in their food an increased lifespan and healthspan was noted in both male and female mice without overt detrimental side effects as noted in the injection group. One of the male mice in the sirolimus (rapamycin) treated cohort survived approximately 1400 days, which the authors of this study believe is likely one of the longest-lived wild type mice of its kind ever reported.11

Studies in humans are underway, but little data exists so far directly on sirolimus and its impact on lifespan and healthspan. A retrospective study looked at off label use of sirolimus in patients (with mainly once weekly use) with a dose ranging from 1 -20mg, and the most common dose being reported as 6mg. The study implemented a survey that found sirolimus users reported high perceived quality of life and good health status but given the nature of the study specific health outcomes were difficult to measure.12

With regards to dosing for longevity, though comparative data is limited, some believe that an intermittent sirolimus dosing schedule could result in fewer adverse events than everyday dosing. For intermittent dosing, some literature suggests that doses of 6mg once weekly are most common for longevity. In limited studies available thus far, daily dosing in humans seems to be less common.12,13 Recently, Dr. Jonathan An, the speaker for our Sirolimus and Aging webinar, was approved to initiate a study on sirolimus (rapamycin) and its impact on periodontal disease. This study will also include a dose finding step, starting at 0.5mg with a potential goal dose of 6mg per week, though, dose escalation is planned to continue beyond the 6mg per week dose.14

The role of Nicotinamide Adenine Dinucleotide (Oxidized)

Nicotinamide Adenine Dinucleotide (NAD) plays an essential role in maintaining cellular redox homoeostasis with oxidized (NAD+) and reduced (NADH) forms existing in a cycle that is essential for energy production in the body. Age related reduction in cellular NAD+ concentrations are hypothesized to play a role in metabolic and aging related disorders.15 Given this link, some have proposed that increasing NAD+ levels may slow or potentially even reverse some aspects of aging or aging related diseases.15 Decreasing levels of NAD+ are thought to be linked to several major hallmarks of aging including loss of proteostatis, deregulated nutrient-sensing, cellular senescence, and mitochondrial dysfunction among others15

An area of particular interest is the impact of NAD on cognitive impairment or cognitive decline. In vitro and in vivo data in mice has shown the potential for NAD 250mg/kg intraperitoneal injection to improve cognitive function and reduce neuroinflammation via mitochondrial protection and reduction of reactive oxygen species.16 One study of NAD 10mg/kg nasally administered to rats 2 hours after ischemic brain injury found significantly reduced ischemia-induced neurological deficits.17 Follow up studies on 20mg/kg intranasal dosing administered after induced brain injury in rats have also found reduction in traumatic brain injury induced neuronal death.18 Mouse models have also demonstrated a link between NAD+ levels in the brain and improvements in learning and memory in Alzheimer's disease rodent models.19

Combination Therapy

In addition to potential benefit of these agents alone, some studies are looking at evaluation of combination products such as the use of metformin and sirolimus either concomitantly or as part of a schedule for improving lifespan, preventing cancer, and management of other age-related pathogeneses such as atherosclerosis.20,21 One study in mice evaluated mice from ages 12 to 30 weeks fed a control diet or diets supplemented with sirolimus, metformin, or a combination of both. The study found that sirolimus alone reduced weight gain, adiposity, and inflammation but exacerbated hyperglycemia and hypertriglyceridemia. Metformin alone reduced hyperinsulinemia and c-reactive protein (a marker of inflammation) but exacerbated nephropathy. The study noted a combination of both resolved some of the adverse effects of either treatment, by reversing the effects of sirolimus on hepatic insulin resistance and normalizing insulin sensitivity. Metformin

was also noted to mitigate hyperglycemic and hypotriglyceridemic effects of sirolimus. The sirolimus was found to attenuate expression of genes in adipose tissue related to adipose tissue expansion, inflammation, and cell senescence.22 One retrospective study of patients taking combination sirolimus and metformin vs sirolimus alone vs metformin with other immunosuppressants looked at the impact of these treatments on overall survival and disease-free survival in patients who received a liver transplant for hepatocellular carcinoma related to hepatitis B. The study found patients treated with combination sirolimus and metformin had significantly longer survival and disease-free survival than other groups.23 More randomized clinical controlled trial data is needed as well as studies that analyze adverse events between single and combination agent treatment groups. Combination therapy offers an interesting potential future avenue for study, though, human use safety and efficacy data is still needed on these combination regimens.

Summary

Aging related disease is a major cause of morbidity and mortality globally. Currently, clinical trials are underway evaluating a variety of different active pharmaceutical ingredients, such as sirolimus, metformin, and NAD+ for their impact on various hallmarks of aging and age-related diseases. To learn more, sign up for our webinar on Sirolimus (Rapamycin) and Aging with expert Dr. Jonathan An Sirolimus (Rapamycin) and Aging - WebinarNinja or, after July 3rd, access the replay on our online learning management site.

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