Clinical Review:

Pharmacotherapy for Management of Aging, Improving Healthspan, and Decreasing Cognitive Decline

Introduction

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 including[1]:

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 test subjects. 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. The researchers noted that the beneficial impacts on life expectancy of the 3-month treatment was similar to benefits with lifelong use in other studies.[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 others[15]

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] Animal studies have also demonstrated a link between NAD+ levels in the brain and improvements in learning and memory in Alzheimer's disease rodent models.[19]

Though not yet studied in combination, NAD+ is sometimes administered nasally with B12 for prevention of cognitive decline. Vitamin B12 deficiency has been linked to neurological damage including muscle weakness and cognitive decline.[28] One study evaluated nasal B12 for maintenance of appropriate B12 level in elderly patients. The study had patients with B12 levels <250pmol/L and hyperhomocysteinemia (>15mmol/L) or two or more symptoms related to B12 deficiency apply 1000mcg cyanocobalamin nasally (500mcg per nostril). The treatment was administered once daily for 14 days followed by weekly administration or 1000mcg every three days with no loading dose period. The study found both dosing regimens to be effective for replenishing vitamin B12 levels.[28]

Methylene Blue and Cognitive Decline

Methylene blue is commercially available as an injectable that has been FDA approved to treat cyanide poisoning,

carbon monoxide poisoning, and methemoglobinemia. In recent years studies have begun to evaluate the potential benefit of methylene blue off label for a variety of conditions, including those associated with cognitive dysfunction such as Alzheimer's Disease, Parkinson's Disease, and brain injuries. Methylene Blue is thought to have benefit for these conditions via its stabilizing effect on **mitochondrial function** and its effect on reduction of reactive oxygen species. Mitochondrial dysfunction and oxidative stress are often implicated in progressive neurodegenerative disorders such Alzheimer's disease and Parkinson's disease and in damage secondary to TBI.[25,26] Additionally, new research both in vitro and in mice has suggests that methylene blue may reduce neuronal apoptosis and improve blood-brain barrier integrity.[27]

One recent double-blind, placebo-controlled trial on methylene blue and aging aimed to evaluate the effect of 2 week and 12-week administration of methylene blue on cerebral blood flow, functional connectivity, memory and attention cognitive abilities in healthy patients, those with mild cognitive impairment, and those with Alzheimer's Disease. Patients were given methylene blue 282mg orally daily and evaluated at 2-week and 12-week timepoints. This study has not yet undergone peer reviewed publication, but available results show methylene blue treated patients in the mild cognitive impairment group to score better on tests such as Face-Name Task. The difference was not noted in patients in the healthy aging group.[29]

Another dose finding study evaluated doses of 69, 138, or 228mg/day in patients with mild to moderate Alzheimer's Disease. By week 24, patients with moderate Alzheimer's Disease had significant improvements in the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog). By week 50 patients with both mild and moderate disease had improvement in ADAS-cog score. The authors conclude that 138mg is the minimum safe and effective dose in Alzheimer's Disease.[30,31]

GHK-Cu (Copper Peptide)

Copper peptide (GHK-Cu or glycyl-lysine-histidyl copper complex) has been of interest for topical use for aging for years. Studies on topical use have evaluated concentrations ranging from 0.2-2%.[33,34] More recently there has been renewed interest on GHK-Cu for cognitive decline in aging. Though the exact mechanism of action has not been fully elucidated, GHK-Cu may work against aging due to antioxidant effects. One study in mice evaluating GHK to GKH-Cu to copper alone noted that GHK-Cu was more effective than GHK alone in terms of antioxidant effect, and copper alone produced no benefit.[35,36] It has also been noted that endogenous GHK declines with age, with higher levels in young healthy people as compared to older adults around age 60.[37]
One study of GHK-Cu dosed at 15mg/kg nasally daily for 8 weeks in mice as compared to placebo (saline). Mice were given spatial memory and learning navigation tasks and were monitored for neuroinflammation and axonal damage. Mice in the GHK-Cu group had an enhanced performance on special memory tests and learning navigation tasks and demonstrated decreased neuroinflammation and axonal damage markers as compared to the placebo group.[35] In addition to studies on general aging, animal trials in mice have also evaluated the potential for GHK-Cu in mice with Alzheimer's Disease. One study evaluated 15mg/kg GHK-Cu given three times weekly for 3 months (treatment started in 4-month-old mice and given until 7 months of age). The intranasal GHK-Cu treatment was found to delay cognitive impairment, reduce amyloid plaques, and lower inflammation levels in the frontal cortex and hippocampus.[38] Randomized controlled trials in human patients are needed to confirm the benefit of GHK-Cu for cognitive decline and Alzheimer's Disease as well as to evaluate safe and effective human dosing, but preliminary animal trials are promising.

Antioxidants

Recent studies have also evaluated glycine and N-acetylcysteine supplementation for improving lifespan and healthspan. Oxidative damage is a major contributing factor to **mitochondrial dysfunction** and glycine and n-acetylcysteine supplementation has been demonstrated to correct deficient glutathione synthesis and lower levels of reactive oxygen species.[39] One clinical trial evaluated 100mg/kg/day of glycine and N-acetylcysteine (GlyNAC group) vs placebo (200mg/kg/day alanine). After 16 weeks of treatment the GlyNAC group showed improvement in markers of the hallmarks of aging including improved insulin resistance and improved fasting insulin (**dysregulated nutrient sensing**) and improved **mitochondrial function** among others. Though one of the major mechanisms of this combination is maintaining healthy levels of glutathione, researchers noted that supplementation of these precursors improved markers of aging whereas supplementation with glutathione itself did not.[39] Further data on the role of supplementation of these precursors and on glutathione is needed as human trials for this combination remain small. Additionally, compared to other pharmaceuticals studied for mitigation of aging, relatively large doses are needed of these precursors, which makes supplementation a challenge.
Resveratrol, a molecule found in fruits and vegetables including grapes, blueberries, cucumbers, and tomatoes among others, is another antioxidant under evaluation for its impact on aging and age-related disease. Resveratrol is thought to reduce oxidative stress, improve **mitochondrial function**, and regulate apoptosis.[44] Studies on resveratrol for longevity are currently mainly in mice, but doses ranging from 20-200mg/kg have been studied in mice and demonstrated prolonged longevity and improved cognitive impairment.[44] Currently, data on human use for aging and age-related diseases are limited. Most studies evaluate resveratrol orally at a wide range of doses between 10 and 500mg per dose. Randomized placebo-controlled trials have demonstrated some improved endothelial function with resveratrol supplementation at 100-300mg daily dosing. Resveratrol supplementation has also been evaluated for impact on sirtuin1 (SIRT1), a molecule that is cardioprotective as well as protective for aging related disease. One study on resveratrol 500mg per day up to 3g per day (in three divided doses) as tolerated found significant increased expression of SIRT1 as compared to placebo.[45] Another study on resveratrol 75mg twice daily for 12 months in postmenopausal women found significant improvement in cerebrovascular responsiveness as measured by cognitive tasks such as verbal memory tasks as compared to placebo.[46] Limited studies have also evaluated resveratrol for Alzheimer's Disease. One placebo-controlled trial evaluated 500mg resveratrol vs placebo once daily for 52 weeks in patients with mild or moderate Alzheimer's disease. Patients in the resveratrol group had a decrease in matrix metallopeptidase 9 of 46% compared to placebo, indicating a potential neuroprotective effect of resveratrol.[47] Additional human trials to further clarify a potential neuroprotective effect and optimal dosing are needed.

Gut Microbiome and Probiotics

Emerging evidence suggests that the gut microbiome plays a critical role for maintaining health and preventing a variety of different disease states. The intestinal microbiome is essential for regulating the pH of the body, reducing the risk of infection, and preserving the integrity of the intestinal lining among other important roles.[40] Though the authors did not have enough information to draw conclusions, studies on sirolimus in mice for mitigation of aging have noted changes in gut microbiome associated with sirolimus use, indicating that the impact of sirolimus on microbiota could be one factor for its benefit for improving lifespan.11 Changes in gut microbiome have also been linked with some other antiaging therapies including metformin. Some researchers hypothesize that the impact of some of these antiaging treatments on increasing diversity of the gut microbiome or increasing the number of good bacteria in the gut may be a factor in their impact on healthspan and lifespan.[42] One randomized double-blind, placebo-controlled trial in older adults evaluated the impact of Bifidobacterium longum $5x10^{10}$ CFU vs placebo for 8 weeks on cognitive impairment. The study used the Repeatable Battery for the Assessment of

Neuropsychological Status (RBANS) as a marker of cognitive function. A statistically significant increase in RBANS was noted after 8 weeks of treatment over the placebo group.41 Other probiotics such as Lactobacillus rhamnosus GG have also been evaluated for their impact on cognitive function. A double-blind, placebo-controlled, randomized clinical trial of adults between the ages of 52-75 evaluated 10 billion CFU Lactobacillus rhamonsus GG twice daily vs placebo. The study found improvements in total cognition score in patients with cognitive impairment, but no difference was noted in patients with intact cognitive function as compared to the placebo group.[43]

Though many studies postulate a link between microbiome and healthspan, data and studies are still in the preliminary stages, and more information on the utility of specific strains and doses are needed.

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.

Formula ID	Formula Title
FA-24039	Sirolimus 6mg Capsule (DiluCap PSD)
FA-23047	Nicotinamide Adenine Dinucleotide (NAD+) 50mg/mL Nasal Spray (Preserved)
FA-23000	Nicotinamide Adenine Dinucleotide (NAD+) 50mg/mL - Methylcobalamin 2mg/mL Nasal Spray Solution
FA-14250	Nicotinamide Adenine Dinucleotide (NAD+) 300mg/mL Nasal Spray
FA-23023	Nicotinamide Adenine Dinucleotide (NAD+) 200mg/mL Sterile Solution (Preserved)
FA-23384	Methylene Blue 100 mg Capsules (#1)(DiluCap SLD)
FA-23387	Methylene Blue 200 mg Capsules (#1)(SimpleCap)
FA-23373	Methylene Blue 50 mg - Ascorbic Acid 75 mg Capsules (#3)(DiluCap SLD)

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