CellPress

Cell Metabolism
Essay

Metformin as a Tool to Target Aging

Nir Barzilai,[1,*] Jill P. Crandall,[1] Stephen B. Kritchevsky,[2] and Mark A. Espeland[2]
[1]Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[2]Wake Forest Older Americans Independence Center and the Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA
*Correspondence: nir.barzilai@einstein.yu.edu
http://dx.doi.org/10.1016/j.cmet.2016.05.011

Aging has been targeted by genetic and dietary manipulation and by drugs in order to increase lifespan and health span in numerous models. Metformin, which has demonstrated protective effects against several age-related diseases in humans, will be tested in the TAME (Targeting Aging with Metformin) trial, as the initial step in the development of increasingly effective next-generation drugs.

Introduction

Over the past decades, remarkable progress has occurred in the science of aging in model organisms. Studies have demonstrated that genetic pathways modulate healthy lifespan in diverse species across great evolutionary distance and established that aging-related pathways constitute a target for intervention (Barzilai et al., 2012; Longo et al., 2015). Lifespan has been verifiably modulated by genetic, pharmacologic, and dietary interventions in multiple model systems.

With support from an R24 grant from the NIA (J. Kirkland, N.B., S. Austad), we gathered gerontologists with expertise in the biology of aging and in clinical geriatrics to discuss ways to target aging in humans. This effort resulted in the design of the study "Targeting Aging with Metformin" (TAME). This trial has been under reviews through several funding mechanisms and has received planning funding from the American Federation of Aging Research. An intended consequence of this effort is to create a paradigm for evaluation of pharmacologic approaches to delay aging. The randomized, controlled clinical trial we have proposed, if successful, could profoundly change the approach to aging and its diseases and affect healthcare delivery and costs. If TAME demonstrates that metformin modulates aging and its diseases, beyond an isolated impact on diabetes, it would pave the way for development of next-generation drugs that directly target the biology of aging. Here, we summarize the major reasons why metformin was chosen to initiate this research.

Targeting Health Span

Interventions that target aging pathways are capable of dramatically extending lifespan and, most importantly, health span, the period of life during which an individual is fully functional and free of chronic illness. There is overwhelming evidence that single gene mutations in nutrient-sensing pathways, such as insulin/insulin-like growth factor (IGF) signaling (Bartke et al., 2001) or the mechanistic target of rapamycin (mTOR) signaling pathways, extend lifespan and health span in invertebrates. More importantly, these pathways have been evaluated in mammalian models, in which health span and lifespan have been extended by genetic manipulation or drugs (Johnson et al., 2013). This raises hope for new interventions, including drugs that slow the aging process and slow the appearance of age-related disease by modulating conserved pathways of aging, as further discussed and developed in recent reviews (de Cabo et al., 2014; Fontana and Partridge, 2015; Fontana et al., 2010).

Interventions to Prolong Lifespan

Recognizing that aging can be targeted, the NIH developed the NIA Interventions Testing Program (ITP). The ITP tests diets, drugs, or other interventions to see if they prevent disease and extend lifespan in genetically heterogeneous (outbred) mice (http://www.nia.nih.gov/research/dab/interventions-testing-program-itp). This program is conducted at multiple centers in order to control for laboratory-specific environmental differences, and testing is done in both male and female animals (Miller et al., 2007; Nadon et al., 2008). Major findings of the ITP include that nordihydroguaiaretic acid and aspirin each increase lifespan of male mice (Strong et al., 2008) and acarbose and 17-α-Estradiol extend mouse lifespan preferentially in males (Harrison et al., 2014). Studies of rapamycin (an mTOR inhibitor) have established the most compelling evidence for targeting aging. When rapamycin is administered late in life, it extends lifespan (Harrison et al., 2009; Miller et al., 2011), slows aging in a dose-dependent manner, shows differential effects by sex (Wilkinson et al., 2012), and is synergistic with metformin.

Metformin Modulates the Biology of Aging and Health Span in Model Organisms

Metformin is a drug approved to treat diabetes but appears to target a number of aging-related mechanisms. Some mechanisms are relevant to glucose metabolism, but with respect to aging these may not be the most important ones. Metformin's multiple aging-relevant actions at the cellular and organismal levels are depicted in Figure 1. Specifically for aging, metformin leads to decreased insulin levels, decreased IGF-1 signaling (Liu et al., 2011), inhibition of mTOR (Kickstein et al., 2010; Nair et al., 2014; Pérez-Revuelta et al., 2014), inhibition of mitochondrial complex 1 in the electron transport chain and reduction of endogenous production of reactive oxygen species (ROS) (Batandier et al., 2006; Bridges et al., 2014; Zheng et al., 2012), activation of AMP-activated kinase (AMPK) (Cho et al., 2015; Duca et al., 2015; Foretz et al., 2010; Lien et al., 2014; Lu et al., 2015; Zheng et al., 2012), and reduction in DNA damage (Algire et al., 2012). Metformin favorably influences metabolic and cellular processes closely associated with the development of age-related conditions, such as inflammation (Saisho, 2015), autophagy (Song et al., 2015; Xie

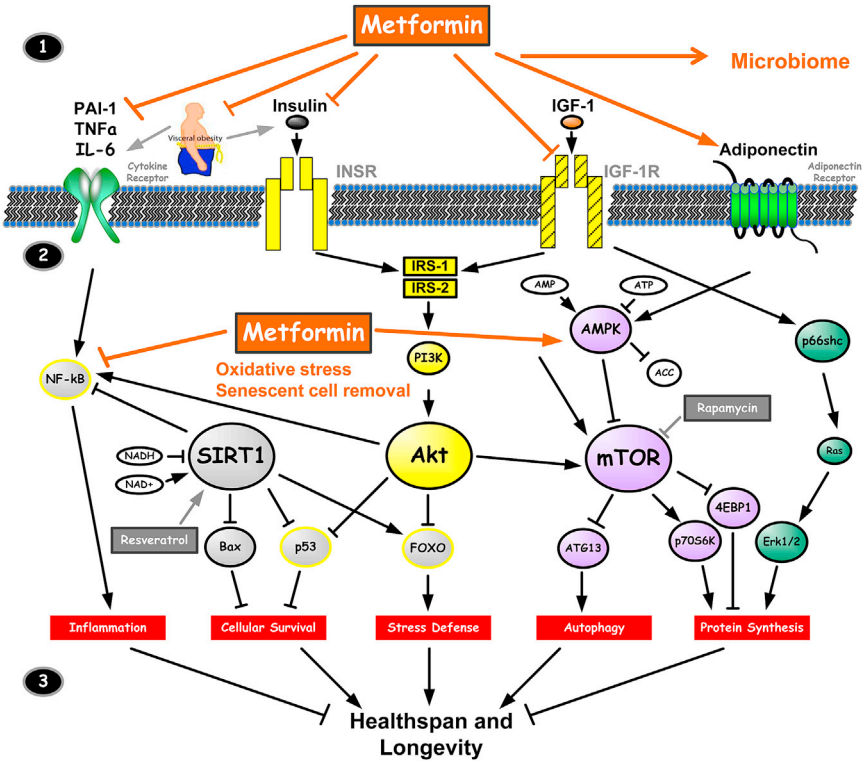

Figure 1. Metformin Targets Multiple Pathways of Aging

The figure depicts schematically the current consensus within the biology of aging community as to pathways that are important in order to target aging and indicates at which points metformin has been shown to have effects (see text). Key take-away: outside of the cell (1, top), metformin has been shown to affect the receptors for cytokines, insulin, IGF-1, and adiponectin, all pathways that are activated with aging and, when modulated, are associated with longevity. (1) Intracellular (2, middle) metformin inhibits the inflammatory pathway and activates AMPK, increasing inhibition of mTOR, which seems to be a major target to modulate aging. Through some of these mechanisms, it also modulates oxidative stress and removes senescent cells (the mitochondrial pathways are not shown, and the mechanisms by which metformin induces senescent cell removal remain unclear). (2) These processes jointly (3, bottom) affect inflammation, cellular survival, stress defense, autophagy, and protein synthesis, which are major biological outcomes associated with aging/longevity. Adapted from Barzilai et al. (2012).

et al., 2011), and cellular senescence (Jadhav et al., 2013; Moiseeva et al., 2013). In *C. elegans* metformin extends lifespan by several possible mechanisms including the alteration of the microbiome, specifically by changing microbial folate and methionine metabolism (Cabreiro et al., 2013). To date, there is no evidence for such effects in humans. Also, other investigators have suggested additional mechanisms for metformin actions (De Haes et al., 2014; Onken and Driscoll, 2010) supporting widely pleotropic effects.

It is currently unclear whether metformin has multiple effects on multiple pathways or whether its observed effects reflect downstream consequences of a primary action on a single mechanism of aging. For example, an attractive explanation suggests (Foretz et al., 2014) that the primary action of metformin is to inhibit mitochondrial complex 1. This inhibition may have multiple downstream effects, but importantly, it would lead to a change in the AMP/ATP ratio, which then activates AMPK. This activation maybe relevant to metformin's known effect on hepatic glucose production (through decreased gluconeogenesis), but it also may suppress lipid synthesis and exert insulin-sensitizing effects, resulting in decreased plasma insulin levels and decreased mTOR activity. However, it is also possible that the singular effect of metformin has not yet been identified, and therefore metformin's mechanisms of action are worth further investigation.

Beyond these cellular processes, there is a growing body of evidence that metformin can delay aging and increase healthy lifespan in vivo, specifically in nematodes and several rodent strains by adding metformin to the diet (Anisimov et al., 2008, 2011; Cabreiro et al., 2013; De Haes et al., 2014). It increases mean lifespan in female outbred mice by ~40% (Anisimov et al., 2008). When started early in life, mean lifespan was increased by 14%, but with initiation at older ages, this effect declined (Anisimov et al., 2011). Metformin delays the onset of carcinoma and extends lifespan by a mean of 8% in a breast cancer model (Anisimov et al., 2010), and extends lifespan by ~20% in a model of Huntington's disease (Ma et al., 2007) only in males. A more recent study (Martin-Montalvo et al., 2013) demonstrated that metformin increased lifespan by 4%–6% in different mouse breeds. The effects on health span indices such as time on rotarod, distance on treadmill, open field tests, cataract index, oral glucose tolerance tests, insulin tolerance, and cognitive function (Allard et al., 2016) were improved by ~30%. As expected in these studies, metformin also increased AMPK activity and increased antioxidant protection, resulting in reductions in both chronic inflammation and accumulation of oxidative damage (Martin-Montalvo et al., 2013), all of which may contribute to health span and lifespan seen in animal models.

Not all studies have shown similar effects of metformin on life or health span. Feeding metformin to *Drosophila* resulted in a robust activation of AMPK and reduced lipid stores, but did not increase lifespan (Slack et al., 2012). One possibility is that the dose of metformin in this study was toxic. The dose of 1 mM is well above the comparable dose range in humans, and indeed doses higher than this increased mortality. This is also the case in mammals. When using a 10-fold increase in the dose that showed benefit in mice, mortality increased (Martin-Montalvo et al., 2013). Smith et al. (2010) did not demonstrate increased lifespan in metformin-treated rats, although the high dose used (~15 times the dose used in humans) may have been toxic. Additionally, the investigators used caloric restriction as a positive control and failed to observe the expected increased lifespan.

Human Studies of Metformin that Target Age-Related Diseases

If metformin can target and delay aging, its administration should be associated

with fewer age-related diseases in general, rather than merely the decreased incidence of a single disease. Data from several randomized clinical trials and multiple observational studies provide evidence for such an effect, which would not be expected from glucose lowering alone.

Clinical Trials

The Diabetes Prevention Program (DPP). The DPP was a randomized trial in U.S. adults at high risk for T2DM by virtue of obesity and impaired glucose tolerance (Knowler et al., 2002). Over 3,000 subjects were randomly assigned to placebo, metformin (850 mg twice daily), or a lifestyle-modification program. Metformin reduced the incidence of T2DM by 31% compared to placebo over a mean follow-up of ~3 years and was effective in all age categories in preventing diabetes, defined by HbA1C level, including the ~20% who were age 60 or older at baseline (Knowler et al., 2015). Further, metformin treatment was associated with improvement in cardiovascular disease (CVD) risk factors (Goldberg et al., 2013; Haffner et al., 2005) and subclinical atherosclerosis (coronary artery calcium) in male participants (Goldberg et al., 2015).

The United Kingdom Prospective Diabetes Study. Patients with T2DM allocated to metformin compared with conventional treatment had risk reduction of ~20% ($p = 0.032$) for CVD and 42% ($p = 0.017$) for diabetes-related death (UKPDS Group, 1998). This evidence from UKPDS provides rationale for metformin's designation as first-line therapy for most patients with T2DM.

Other Trials. In the HOME trial of insulin-treated T2DM patients, addition of metformin resulted in 40% reduction (compared with placebo) in a CVD composite after 4 years of follow-up (Kooy et al., 2009). In non-diabetic subjects, the GIPS III study (Lexis et al., 2014) failed to demonstrate the benefit of short-term metformin treatment (4 months) on left ventricular ejection fraction, major adverse cardiovascular events, and mortality in post-myocardial infarction patients, and the CAMERA trial (Preiss et al., 2014) showed no effect of metformin (18 months) on carotid intimal medial thickness.

Observational Studies

The majority of observational data support metformin benefit in CVD, but residual bias and confounding cannot be ruled out (e.g., most studies have been conducted in patients with diabetes and include an active comparator, which could itself be cardio-toxic). Metformin's potential CVD benefits—particularly in the area of primary prevention—remain an active area of research, including an ongoing randomized trial in the UK (The Glucose Lowering In Non-diabetic hyperglycaemia Trial, GLINT, http://www.isrctn.com/ISRCTN34875079; Anfossi et al., 2010; Whittington et al., 2013).

Observational Studies Suggest Metformin Decreases Cancer Incidence

Several epidemiologic studies have shown that metformin use is associated with reduced cancer incidence and mortality (Landman et al., 2010; Lee et al., 2011; Libby et al., 2009; Monami et al., 2011; Tseng, 2012). While one meta-analysis (Stevens et al., 2012) did not show that metformin prevents cancer, a more thorough analysis that included more data and accounted for heterogeneous comparators showed that overall cancer incidence was reduced by 31% and cancer mortality by 34% (Gandini et al., 2014). There is also evidence from studies performed both in vitro and in vivo of metformin's role in attenuating tumorigenesis (Anisimov and Bartke, 2013; Karnevi et al., 2013; Liu et al., 2011; Quinn et al., 2013; Salani et al., 2012; Tosca et al., 2010). The mechanisms proposed relate to reduced insulin levels, improved insulin action, decreased IGF-1 signaling, and activation of AMPK. Numerous ongoing studies are testing the effect of metformin as adjuvant cancer therapy, with a recently published trial showing negative results in advanced pancreatic cancer (Kordes et al., 2015). Although no trials yet have reported effects of chronic treatment on cancer prevention, studies in early-stage cancer or pre-malignancy suggest this may be fruitful (DeCensi et al., 2015).

Association of Metformin with Better Cognitive Function

Emerging evidence suggests that metformin may preserve cognitive function. In the Singapore Longitudinal Aging Study, metformin use was associated with a 51% reduced risk of cognitive impairment (defined by modified Mini-Mental Status Exam score $\leq$ 23), which remained robust to adjustment for vascular and non-vascular risk factors. Further, the lowest risk was seen in those with longer-term (> 6 years) metformin use (Ng et al., 2014). A large observational study of metformin-treated T2DM patients reported lower rates of dementia than in those treated with other diabetes medications (Cheng et al., 2014). One study suggested that T2DM patients treated with metformin had increased risk for poor cognitive performance (Moore et al., 2013); however, it had a number of methodological flaws (Alagiakrishnan et al., 2013) and has not been replicated. In one small clinical trial, T2DM patients with depression (n = 58) were treated with metformin or placebo for 24 weeks (Guo et al., 2014). The metformin group showed improved cognitive performance and reduced depressive symptoms, concurrent with improved glycemic control. In an unpublished trial, non-diabetic subjects (n = 80) with mild cognitive impairment showed significant improvements in some cognitive domains after 12 months of metformin treatment (Luchsinger et al., 2016). No definitive trials have been conducted.

Association of Metformin with Decreased Mortality

A recent study (Bannister et al., 2014) used retrospective observational data from the UK Clinical Practice Research Datalink. Patients with T2DM who were treated with metformin or sulphonylurea (SU) monotherapy were compared to separate age- and sex-matched control groups without diabetes. SU-treated patients had lower survival than both matched non-diabetic controls and metformin-treated diabetic patients. Surprisingly, metformin-treated diabetic patients had survival rates similar to (and, among those age > 70, even better than) their matched non-diabetic control group, despite the fact that the diabetic patients were more obese and had greater comorbidities at baseline. Mortality benefits have also been described in other observational studies and long-term follow-up of the UKPDS cohort, which showed 36% reduction in all-cause mortality in the metformin treatment group ($p = 0.011$) (UKPDS Group, 1998). Not all studies have been positive—for example, an analysis from the Medicare Current Beneficiary Survey showed only a non-statistically significant survival benefit for metformin-treated patients (Tinetti et al., 2015).

Considerations in Designing Human Metformin Trials

Dosing. While metformin can be prescribed at dosages of up to 2,250 mg/day, no further effects of decreasing glucose are noted after 1,600–1,700 mg/day. After a single oral dose, metformin is rapidly distributed to many tissues following partial absorption by the small intestine, but the luminal concentration in the gastrointestinal tract remains high. After a single 1.5 g dose, the peak plasma concentration of 18 mM occurs in 3 hr, with a mean plasma half-life of about 20 hr (Foretz et al., 2014). It is suggested, however, that an equivalent dose for mice would be up to 10-fold higher. Studies on biodistribution of metformin in mice showed accumulation mainly in the gastrointestinal tract, kidney, and liver.

Safety. Metformin has been used with an excellent safety record for over 60 years. Side effects are monitored closely within clinical trials, and the safety of metformin use in DPP/DPPOS was reported on in 2012, when over 18,000 patients-years of follow-up had accrued, and by which time ~20% of the cohort was age 70 or older (mean age ~64). There were no cases of lactic acidosis or significant hypoglycemia (Diabetes Prevention Program Research Group, 2012). Mild anemia occurred in ~12% of metformin-treated participants versus ~8% in the placebo group ($p = 0.04$). Vitamin B12 deficiency occurred in ~7% of metformin group versus 5% in placebo group after 13 years; risk of B12 deficiency increases with duration of use but was not greater in older compared with younger subjects in DPPOS (Lalau et al., 1990). Further, the risk of lactic acidosis appears to be related to renal function, not age per se, and is currently considered to be very low (Aroda et al., 2016).

In the TAME study, we plan to enroll 3,000 subjects, ages 65–79, in ~14 centers across the U.S. Rather than study the effects of metformin on each separate condition, we will measure time to a new occurrence of a composite outcome that includes cardiovascular events, cancer, dementia, and mortality. TAME will also assess important functional and geriatric end points.

If successful, TAME will mark a paradigm shift, moving from treating each medical condition to targeting aging per se. We expect this to facilitate the development of even better pharmacologic approaches that will ultimately reduce healthcare costs related to aging.

ACKNOWLEDGMENTS

The American Federation for Aging Research has supported the TAME initiative. This work was supported by the Nathan Shock Center of Excellence for the Biology of Aging (P30AG038072, N.B.), the Glenn Center for the Biology of Human Aging (Paul Glenn Foundation for Medical Research) (N.B.), grant 1R24AG044396 from the National Institute on Aging (NIA) (PI, Kirkland; co-PI, N.B.), and grant P30 AG021332 from the NIA to the Wake Forest Older Americans Independence Center (S.B.K.).

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