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ADVANTAGES AND DISADVANTAGES OF USING METFORMIN IN AGING RELATED DISEASES

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ABSTRAK

Tujuan review ini adalah untuk mengeksplorasi efek anti-penuaan metformin, yang tidak hanya menurunkan glukosa tetapi juga memperlambat penuaan sel melalui pengaturan molekul sinyal intraseluler dan aktivasi AMPK, serta melindungi dari penyakit terkait penuaan.Aktivasi AMPK oleh Metformin memungkinkannya untuk mengendalikan kondisi inflamasi, meningkatkan status oksidatif, mengatur jalur diferensiasi berbagai sel, dan akhirnya memberikan efek terapeutik positif pada sel-sel ini. Metformin berperan dalam beberapa penyakit sistem kardiovaskular, gangguan neurologis, kanker, dan sindrom fragile X dengan meningkatkan beberapa parameter dalam beberapa kondisi penyakit ini. Metformin memberikan manfaat pada penyakit terkait penuaan, namun masih diperlukan penelitian lebih lanjut dengan metode dan sampel yang bervariasi untuk menilai efektivitas dan mempertimbangkan efek samping metformin pada pasien diabetes dan non-diabetes, serta melibatkan faktor genetik yang terkait dengan metformin.

ABSTRACT

Advantages And Disadvantages Of Using Metformin In Aging-Related Diseases. This review aimed to explore the anti-aging effects of metformin, which lowers glucose, slows down cell aging through regulating intracellular signaling molecules and activating AMPK, and protects against aging-related diseases. The activation of AMPK by Metformin allows it to control inflammatory conditions, improve oxidative status, regulate differentiation pathways of various cells, and ultimately provide positive therapeutic effects on these cells. Metformin plays a role in several cardiovascular diseases, neurological disorders, cancer, and fragile X syndrome by improving several parameters in some of these disease conditions. Metformin provides benefits in aging-related diseases, but further studies with various methods and samples are needed to assess the effectiveness and consider the side effects of metformin in diabetic and non-diabetic patients, as well as involving genetic factors associated with metformin.

INTRODUCTION

The aging process is a progressive multifactorial phenomenon that exhibits different characteristics at the molecular, cellular, and organism levels. Dysregulated nutrient sensing, DNA damage, the accumulation of reactive oxygen species (ROS), telomere attrition, inflammation, autophagy decline, mitochondrial dysfunction and induction of cellular senescence, and stem cell depletion have been identified in aging and play crucial roles during the aging process.[1,2]

Various physiological and pathological stressors trigger cellular senescence and lead to irreversible cell cycle arrest; mitochondrial function deteriorates due to multiple mechanisms, including the accumulation of mitochondria DNA (mtDNA) mutations, deficient proteostasis leading to the destabilization of respiratory chain complexes, reduced turnover of the organelle, and changes in mitochondrial dynamics. This situation compromises the contribution of mitochondria to cellular bioenergetics, enhances the production of reactive oxygen species (ROS), and may trigger accidental permeabilization of mitochondrial membranes, causing inflammation and cell death.[1,2] Genes and proteins participating in the autophagic process are also involved in alternative degradation processes. In contrast, loss-of-function mutations of genes that regulate or execute autophagy have been causally linked to a broad spectrum of cardiovascular infections.[1,2,3]

Aging is considered to be associated with impaired glucose tolerance and type 2 diabetes mellitus, so the prevalence of metabolic syndrome increases with age. However, various aging-related diseases in different organs and systems have their combination of molecular features of aging. In addition, many aging-related diseases are associated with a chronic inflammatory state, often caused by the long-term accumulation of senescent cells in various tissues.[3,4] Senescent cells accumulate in multiple tissues with aging, contributing to low-grade chronic "sterile" inflammation at sites of chronic pathologies. Pro-inflammatory and matrix-degrading factors secreted by senescent cells negatively alter tissue function and structure.[3] Tissue damage-induced cellular senescence recruits immune cells, especially phagocytic cells, by secreting senescence-associate secretory phenotypes (SASP), which also promotes tissue regeneration by stimulating the self-renewal and differentiation of tissue-resident stem/progenitor cells. The remodeling process is completed when phagocytic cells are cleared by senescent cells, resulting in a senescence-clearance-remodeling sequence. However, this sequence can be disrupted by persistent damage or aging, leading to the accumulation of detrimental effects on senescent cells and the induction of chronic inflammation and fibrosis via the persistence of SASP.[4]

Metformin is a biguanide compound that has the effect of lowering blood glucose levels. Metformin was initially of little clinical interest among the biguanide compounds because its potency was low, so its use required high doses to be effective. Metformin inhibits gluconeogenesis in the liver, thereby lowering blood sugar levels. In addition, metformin reduces cardiovascular events and has renoprotective effects. Metformin has also been reported to prolong life span. Although metformin is usually well tolerated, this drug has some side effects in some patients,

including lactic acidosis, gastrointestinal disorders, and vitamin B12 deficiency. However, these side effects improve with dose titration or drug discontinuation.[5] Metformin regulates intracellular signaling pathways by activating AMPK or other pathways to exert protective effects such as anti-inflammatory, analgesic, and antagonistic oxidative stress.[5]

Metformin, as an anti-aging drug candidate, has unparalleled advantages. Regarding the anti-aging mechanism of metformin, the nutrient pathway is a key mechanism by which it lowers blood sugar levels and mediates its anti-aging effects. Metformin reduces the level of AGEs, a marker of aging, by lowering insulin and blood glucose levels and increasing insulin sensitivity. Metformin can induce the production of antioxidant proteins to maintain ROS homeostasis and reduce oxidative stress. Metformin inhibits mTOR activity by activating AMPK. Downregulation of mTOR leads to the activation of autophagy, preventing the accumulation of damaged proteins.[6]

The American Diabetes Association has recommended that metformin be used after initiating insulin therapy (unless contraindicated or not tolerated) to obtain sustained glycemic and metabolic benefits in treating patients with type 2 diabetes mellitus. Metformin is effective, safe, and inexpensive and may reduce the risk of cardiovascular events and death. Metformin is available in an immediate-release dosage form twice daily or an extended-release dosage form, which can be administered once daily. Metformin as first-line therapy benefits HbA1c, body weight, and cardiovascular mortality. The drug is cleared by renal filtration, and very high circulating levels (e.g., due to overdose or acute renal failure) have been associated with lactic acidosis. However, the incidence of this complication is now known to be very rare, and metformin may be safe to use in people with a reduced estimated glomerular filtration rate (eGFR); The FDA has revised the metformin label to reflect its safety in people with eGFR ≥30 mL/min/1.73 m2.[7] Apart from being an oral anti-diabetic, existing studies show that metformin is widely used in several diseases related to aging, including neurodegenerative diseases, cancer and as an anti-aging agent itself, but the results are still inconsistent between one study to another. Although the side effects are relatively tolerable and improve with drug discontinuation, use in aging-related diseases still requires further research.

METHODS

This narrative review article was prepared by reviewing several articles through a search from the PubMed database by entering the keywords aging, metformin, and aging-related disease. This review only includes articles that are available in English.

DISCUSSION

Pharmacokinetics and Pharmacodynamics of Metformin

After oral administration, intestinal absorption of metformin is primarily mediated by the plasma membrane monoamine transporter (PMAT, encoded by the SLC29A4 gene). In addition,

OCT1 (SLC22A1 gene), can facilitate the transfer of metformin into the interstitial fluid. The role of OCT1 and OCT3 in intestinal transport of metformin remains to be defined. Hepatic uptake of metformin is mediated primarily by OCT1 (SLC22A1) and possibly by OCT3 (SLC22A3). Metformin is also a good substrate for multidrug extrusion and human toxin 1 (MATE1, encoded by the SLC47A1 gene) and MATE2-K (SLC47A2 gene)]. MATE1 (SLC47A1) may contribute to the excretion of metformin from the liver and kidney. Metformin is also a good substrate for multidrug extrusion and human toxin 1 (MATE1, encoded by the SLC47A1 gene) and MATE2-K (SLC47A2 gene). MATE1 (SLC47A1) is highly expressed in the liver, kidney, and skeletal muscle and may contribute to the excretion of metformin from the liver and kidney. Metformin is not metabolized in the liver, but drug-drug interactions via inhibition of metformin transporters (OCTs and MATEs) are clinically relevant.[8]

Metformin primarily lowers basal and postprandial plasma glucose by suppressing excessive hepatic glucose production (via reduced gluconeogenesis). Other potential pharmacodynamic roles of metformin include increased glucose uptake, increased insulin signaling, decreased fatty acid and triglyceride synthesis, and increased fatty acid beta-oxidation. Metformin may also increase glucose utilization in peripheral tissues and reduce food intake and intestinal glucose absorption. Because metformin does not stimulate endogenous insulin secretion, it does not cause hypoglycemia. The need to check renal function before and at least annually during treatment with metformin is emphasized to reduce the risk of drug accumulation, which, in turn, reduces the risk of excessively raised lactate concentrations. Checks are suggested more frequently in the elderly or individuals at risk of a rapid decline in renal function. Hemodialysis is warranted if metformin concentrations are markedly raised, and/or renal insufficiency is severe, pH is particularly low, or there is liver disease, sepsis, or another limiting co-morbidity. It is noted that lactic acidosis can occur in patients with diabetes unrelated to metformin therapy, and respiratory (type A) causes require different treatment approaches.[8,9,10,11]

The molecular mechanisms underlying the action of metformin appear to be complex and are still a topic of debate. However, there is general agreement that metformin administration causes AMPK phosphorylation in the liver, which may ultimately account for many of the pharmacological effects of metformin, including inhibition of glucose and lipid synthesis. Metformin also inhibits complex I of the mitochondrial chain, suggesting that this inhibition may activate AMPK by increasing the cellular AMP: ATP ratio. Activation of AMPK by metformin also suppresses the expression of SREBP-1 (SREBF1), a major lipogenic transcription factor. Activated AMPK increases glucose uptake in skeletal muscle through increased glucose transporter 4 (GLUT4) translocation activity (encoded by the SLC2A4 gene). Metformin has also been shown to have tumor-suppressive capabilities and is emerging as a potential agent that protects against cancer by activating AMPK and downregulating cyclin D1.[9,10,11]

There is interindividual variability in response to metformin, and this leads to many drug-drug interactions with metformin. Several transporters are involved in metformin transport, so

metformin pharmacokinetics will change only slightly when only one transporter is inhibited. This means that even potent inhibition of one transporter in vitro may not necessarily cause changes in pharmacokinetics in vivo. Anti-diabetic drugs have largely inhibited OCT1- and OCT2-mediated transport of metformin. To some extent, a large group of cardiovascular agents inhibit metformin transport via OCT1, OCT2, and OCT3. Proton pump inhibitors (PPI) were shown to inhibit metformin absorption via OCT1, OCT2, and OCT3; Trimethoprim showed competitive inhibition of OCT2, MATE1, and MATE2-K. Four different anti-cancer drugs belonging to the group of tyrosine kinase inhibitors have been shown to inhibit the relevant metformin transporters (OCT1, OCT3, MATE1, and MATE2-K).[12]

Effects of Metformin on Several Aging-Related Diseases

Explore how metformin might synergize or conflict with other treatments for aging-related diseases, such as statins or anticoagulants in cardiovascular disorders. Metformin exerts many other beneficial effects independent of its glucose-lowering effects in both experimental animals and human subjects. Among the most prominent effects is protection against a wide range of cardiovascular and related metabolic disorders, including dyslipidemia, diabetic cardiomyopathy, chemotherapy drug-induced cardiotoxicity, ventricular dysfunction, and heart failure. This protective effect is based on metformin's ability to atheroprotection, activation of the AMPK/autophagy pathway, suppression of mitochondrial ROS-mediated pro-inflammatory pathways, myocardial protection, and metformin's activation of Sirtuin signaling.[13,14]

Metformin can regulate the function of macrophages in atherosclerosis, including reducing the differentiation of monocytes and inhibiting the inflammation, oxidative stress, polarization, foam cell formation, and apoptosis of macrophages. The mechanisms by which metformin regulates the function of macrophages include AMPK, AMPK independent targets, and NF-κB.[14] Metformin systematically induces atheroprotective genes in macrophages via AMPK and ATF1, thereby suppressing atherogenesis directly. Moreover, AMP-dependent metformin-mediated macrophage gene regulation is mediated by the transcription factor ATF1, the antioxidant gene HMOX-1, lipid homeostasis genes such as ABCA1, and anti-atherosclerotic eicosanoids.[14]

The beneficial effects of metformin have been demonstrated in various studies on cardiovascular disorders, especially atherosclerosis, myocardial injury, and heart failure (HF). A study evaluated the effect of metformin in reducing cardiovascular risk in diabetic patients with stable angina pectoris and non-obstructive coronary artery stenosis (NOCS), which was divided into 3 allocations: 86 normal blood glucose (NG) subjects, 86 prediabetic subjects, and 86 prediabetic subjects with treated with metformin (pre-DM+metformin dose 850 mg twice daily). Metformin treatment can reduce the high risk of Major Adverse Cardiac Events (MACE) in prediabetic patients by reducing coronary endothelial dysfunction. These results were based on a decrease in the percentage of endothelial LAD dysfunction and a decrease in MACE.[15]

A retrospective cohort study to evaluate the effect of metformin in T2DM patients with renal insufficiency where the primary outcome was MACE including hospitalization due to acute myocardial infarction, stroke, transient ischemic attack, or cardiovascular death. The Propensity score analyses were used to compare MACE's cause-specific hazard between treatments and estimate cumulative risk accounting for the competing risks of changing therapy or noncardiovascular death. Diabetic patients with reduced renal function treated with metformin monotherapy have a reduced risk of MACE compared with sulfonylureas.[16] A population-based cohort study revealed that metformin adherence in patients with T2DM who required a first-line treatment may reduce the risk of subsequent CVD. Despite the availability of numerous novel anti glycemic agents, metformin adherence by patients who require a combination of anti glycemic agents provides an additional benefit of CVD protection.[17]

An RCT study tested the effects of metformin in patients with left ventricular hypertrophy (LVH) who had coronary artery disease (CAD), with insulin resistance (IR) and/or prediabetes. A total of 68 patients; mean age 65 ± 8 years) without diabetes who had CAD with IR and/or prediabetes were randomized to receive metformin XL (daily dose 2000 mg) or placebo for 12 months. The primary outcome was a change in left ventricular mass index (LVMI) relative to body height. Metformin treatment significantly reduced LVMI, LVM, systolic blood pressure, body weight, and oxidative stress.[18] Metformin users showed significantly lower rates of MACE in PDM than users who had never used metformin. PDM users who never used metformin showed higher levels of SGLT2 and leptin in pericoronary fat than metformin users. Metformin improves cardiovascular outcomes by reducing inflammatory parameters, SGLT2, and leptin levels and ultimately increasing SIRT6 levels in AMI-PDM patients undergoing CABG.[19]

Metformin and Neurological Disease

Diabetes and prediabetes can lead to the development of dementia due to the complex relationship between glucose metabolism and cognitive impairment of the nervous system. The mechanisms involved include mitochondrial dysfunction, inflammation, oxidative stress, protein glycation, autophagy, and intracellular signal transduction pathways, impairing protein phosphorylation, insulin signaling, and glucose homeostasis in the nervous system. Inadequate glycemic management can increase the risk of dementia and cognitive decline. Duration of diabetes, increasing age, increasing HbA1c, and the presence of diabetic retinopathy are strong risk factors for worsening diabetic peripheral neuropathy. Metformin reduces the formation of pro-inflammatory cytokine 1β, and increases neurotrophic and angiogenic factors, while the upregulation of TNF-α is not affected by metformin.[20]

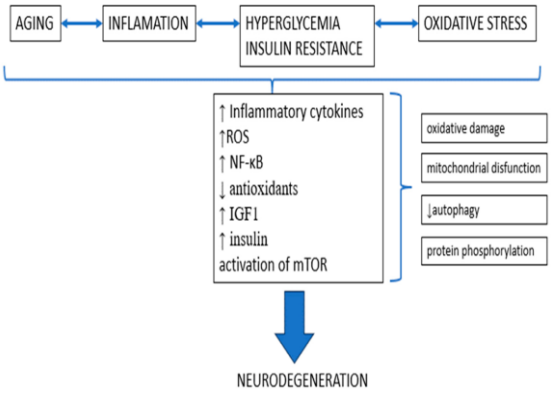

Figure 1. Source :Sardu et al, 2021.

A prospective observational study showed that the use of metformin monotherapy may reduce the risk of cognitive dysfunction in individuals with type 2 diabetes.[21] A meta-analysis including 10 studies (9 retrospective cohort studies and one prospective cohort study) involving 254,679 patients showed that Metformin use is associated with a significant reduction in cognitive impairment in patients with type 2 diabetes.[22] A meta-analysis released in 2020, analyzing data from more than 1 million patients, showed that specific anti-diabetic agents such as metformin and DPP-4 inhibitors may reduce the risk of dementia compared with insulin use.[23] Another meta-analysis suggests that metformin may improve cognitive function in diabetes patients, thereby potentially improving the clinical conditions of mild cognitive impairment and Alzheimer's dementia. However, its use for the prevention of dementia in non-diabetic older adults is not currently recommended.[24] However, another meta-analysis study showed that metformin did not show a significant impact on improving cognitive function or providing protection against all forms of dementia, including vascular dementia and Alzheimer's dementia.[25]

The effect of metformin on neurological disorders was found in a different study of 49,705 type 2 DM patients in China. Metformin use is associated with an increased risk of diabetic peripheral neuropathy and increases with increasing metformin dose. Metformin is a major risk factor for diabetic peripheral neuropathy in young patients aged less than 60 years. Giving vitamin B12 and metformin can reduce the risk of diabetic peripheral neuropathy.[26] The role of metformin in Parkinson's disease in type 2 DM patients is related to dose and intensity of use. This cross-sectional study involved people with type 2 DM and categorized them as metformin users or non-users who were followed up for 3 and 5 years. Patients who receive low doses and intensity of metformin have a lower risk of developing Parkinson's disease, while higher doses and intensity of metformin do not have a neuroprotective effect.[27]

Long-term metformin users have serum vitamin B12 concentrations that are deficient or borderline low. The mechanism by which vitamin B12 deficiency may lead to and/or exacerbate peripheral neuropathy in patients with T2DM treated with long-term metformin therapy remains to be explored. Metformin interferes with vitamin B12 absorption in the gastrointestinal tract. Metformin-induced vitamin B12 deficiency may have a deleterious impact on the macrovascular

system, including hematological and neurological manifestations, via increased concentrations of methylmalonic acid and homocysteine.[28]

A meta-analysis aims to explore the effect of metformin use on vitamin B12 deficiency in patients with type 2 diabetes mellitus (T2DM). Factors significantly associated with vitamin B12 deficiency in patients with T2DM and receiving metformin include the duration of metformin use and a greater dose. Vitamin B12 in nutrition is important in patients receiving metformin because metformin-induced B12 decrease may harm patients with T2DM. Supplemental vitamin B12 may be advantageous for those on metformin. Besides that, it is important to test vitamin B12 levels in patients receiving metformin, even if patients are asymptomatic, in order to identify deficiency earlier and prevent complications.[29]

The protective effect of vitamin B12 supplementation on developing or slowing diabetic peripheral neuropathy due to long-term exposure to metformin has not yet been determined. A meta-analysis found that vitamin B12 can improve neuropathic symptoms and reduce pain in patients with diabetic neuropathy. This meta-analysis showed that patients who received B12 alone or in combination with other drugs experienced a more significant reduction of mean neuropathic symptoms than placebo. In addition, they experienced more significant pain reduction scores compared to patients who received a placebo. Vitamin B12 occurs in different forms, and it is called cobalamin. The main form of cobalamin utilized in vitamin supplements is cyanocobalamin, while methylcobalamin is a coenzyme form that is a crucial cofactor for the function of vitamin B12-dependent methyltransferases.[30]

The effect of vitamin B12 in improving diabetic neuropathy symptoms was also confirmed in a study conducted by Didangelos et al., which showed that the treatment of patients with diabetic neuropathy with 1 mg of oral methylcobalamin for twelve months increased plasma B12 levels and improved all neurophysiological parameters, sudomotor function, pain score, and quality of life. The analgesic action of B12 is possibly mediated by an increase in the availability and effectiveness of noradrenaline and 5-hydroxytryptamine in the descending inhibitory pain modulation system (endogenous opioid system). Methylcobalamin acts on the synthesis and regeneration of myelin, enhances nerve conduction, and decreases neurotransmitter levels.[31]

Metformin and anti-aging

Administration of metformin increases health span and lifespan in experimental animals, and evidence from clinical trials and observational studies suggests that metformin delays various age-related morbidities. Although metformin has been shown to modulate several biological pathways at the cellular level, the pleiotropic effects of metformin on human aging biology have not been thoroughly studied.

Metformin at low doses can prolong the life of diploid fibroblasts and mesenchymal stem cells. One of the causes of cellular aging is a decrease in the expression levels of glutathione peroxidase 7 (GPx7). Low levels of GPx7 cause cellular senescence to occur prematurely. Metformin

increases levels of nuclear factor–erythroid 2–related factor 2 (Nrf2), binding to the antioxidant response element in the GPx7 gene promoter to induce its expression. Metformin has significant metabolic and non-metabolic association effects with aging, including pyruvate metabolism and deoxyribonucleic acid (DNA) repair in muscle, peroxisome-proliferator-activated receptor (PPAR) and sterol-regulatory element-binding protein (SREBP) signaling, acid oxidation. Mitochondrial fat, and collagen trimerization in adipose tissue. Various mechanisms of action of metformin on anti-aging include the autophagy pathway with AMPK activation and mTOR inhibition, increased antioxidants, ROS inhibition, inhibition or improvement of mitochondrial function, and inhibition of inflammation.[32]

The Metformin in Longevity Study (MILES) was a double-blind crossover clinical trial study in 14 elderly participants with impaired glucose tolerance to determine whether metformin (1700 mg/day) could restore youthful gene expression and transcriptomic changes in muscle and adipose tissue after 6 weeks of treatment. The results of the MILES study show that metformin modulates metabolism and non-metabolic gene expression related to aging (modifying several pathways associated with aging, including metabolic pathways, collagen trimerization and extracellular matrix (ECM) remodeling, adipose tissue and fatty acid metabolism, mitochondria).[33]

Patients aged ~70 years (n = 14) were given metformin and placebo each for 6 weeks in a randomized, double-blind, placebo-controlled crossover trial. Metformin therapy decreased 2-hour pp glucose, insulin AUC, and insulin secretion compared with placebo. Using FDR<0.05, 647 genes were differentially expressed in muscle, and 146 genes were differentially expressed in adipose tissue. Metabolic and non-metabolic pathways were significantly affected, including pyruvate metabolism and DNA repair in muscle and PPAR and SREBP signaling, mitochondrial fatty acid oxidation, and collagen trimerization in adipose.[34] Drug target study Mendelian randomization, observational cross-sectional design assessed the specific effects of metformin (AMPK, ETFDH, GPD1, and PEN2). Biomarkers of aging are phenotypic age (PhenoAge) and leukocyte telomere length. AMPKγ2 (PRKAG2)-induced decrease in HbA1c was associated with younger PhenoAge but was not associated with leukocyte telomere length. Metformin may promote healthy aging through targeting GPD1 and AMPKγ2 (PRKAG2), and the effect may be partly due to its glycemic properties.[35]

Metformin and Cancer

Cancer occurs due to abnormal and uncontrolled development of cells and can attack tissues around the body. Typical characteristics include: 1) signals of excessive growth; 2) failure to respond to anti-growth signals; 3) unscheduled cell death; 4) unlimited proliferation potential; 5) angiogenesis; and 6) tissue invasion and metastasis so that cancer cells can spread through the bloodstream or lymph to other parts of the body. Diabetes is a common disease that can occur throughout human life and can be a factor in the occurrence of various types of cancer. Also, people with diabetes are more likely to develop various types of cancer. The incidence of various types of cancer is higher in T2DM patients due to insulin resistance and mitogenic effects caused by

hyperglycemia. Preclinical studies have shown that metformin has anti-cancer properties and acts additively or synergistically when combined with anti-cancer agents.[36]

The mechanism underlying the diabetes-cancer relationship is insulin resistance, which causes secondary hyperinsulinemia. Insulin can exert mitogenic effects via the insulin-like growth factor 1 (IGF-1) receptor. Metformin can reduce circulating insulin levels and activate the immune system at the extracellular level. Intracellularly, metformin can activate the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) pathway via the human epidermal growth factor receptor-2 (HER2) pathway, inhibiting several STAT3-related signaling pathways, including IL-6/JAK2/STAT3 signaling, inhibits protein synthesis, induces cell cycle arrest and apoptosis, and reduces IGF-1 and insulin-mediated signals. Metformin exhibits anti-cancer actions through direct and indirect effects: indirect effects include reduction of circulating glucose and insulin levels and suppression of tumor progression by indirectly modulating IGF-1 signaling, which promotes tumor cell growth, whereas direct effects include cell cycle inhibition, suppression of epithelial-mesenchymal transition (EMT), and inhibition of tumorigenesis and cancer progression.[37,38,39]

A meta-analysis of randomized clinical trials to evaluate the effects of metformin added to systemic anti-cancer therapy in patients with advanced or metastatic cancer. Patients cancer with chemotherapy receiving metformin did not increase tumor response, compared with those without metformin. The overall effects of metformin in patients with advanced or metastatic cancer receiving chemotherapy did not provide clinical benefits according to parameters like progression-free survival/PFS and overall response rate/OS.[40] Secondary analysis of a phase III randomized, double-blind trial in early breast cancer showed metformin did not reduce the risk of new cancer development in non-diabetic patients with breast cancer.[41] ALTO RCT study in HER2-positive breast cancer patients randomized to receive trastuzumab, lapatinib combination both for 1 year. This study analyzes the relationship between metformin use and improved clinical outcomes in diabetes patients with HER2-positive primary breast cancer. Diabetic patients not treated with metformin experienced worse DFS. Metformin may improve the poor prognosis associated with diabetes and insulin treatment, especially in patients with HER2-positive and hormone receptor-positive primary breast cancer.[42]

Metformin in Fragile X syndrome

Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability and the most commonly known genetic cause of autism spectrum disorders. Children with FXS experience behavioral disturbances and sleep problems, anxiety, inattention, learning difficulties, and speech and language delays. Until now, there is no suitable therapy for FXS. However, there are several interventions and treatments aimed at managing symptoms and improving the quality of life for individuals with FXS. A combination of non-pharmacological therapy and pharmacotherapy is the current effective treatment for FXS. Some promising pharmacotherapy modalities for FXS include metformin, sertraline, and cannabidiol. Gene therapy targeting the FMR1 (Fragile X Messenger

Ribonucleoprotein 1) gene mutation that causes FXS has potential as a prospective treatment of FXS in the future.[43]

Many studies have focused on behavioral and cognitive problems in young patients with FXS, but there have been no studies on aging problems in FXS patients. Discovery of Fragile Apart from FXTAS, neurobiological changes in FXS also cause Parkinson's disease and other movement disorders, especially in long-term atypical anti-psychotic users. Approximately 40% of men with FXS have increased levels of FMR1-mRNA, thereby increasing the risk of experiencing FXTAS. The neurodegeneration seen in aging premutation carriers with FXTAS is thought to be caused by increased FMR1 mRNA levels that do not usually occur in those with FXS. Apart from neurodegenerative disorders, some adult men suffering from FXS also experience obesity, hypertension, and impaired kidney function.[44]

Metformin is mainly used for the treatment of diabetes. Metformin also has anti-cancer properties and has beneficial effects on several neurological conditions in Alzheimer's Dementia, depression, and FXS. Metformin plays a role in regulating mTOR and AMPK signaling pathways and potentially has a range of direct and indirect neuroprotective effects. Metformin can normalize hyperactive conditions of mTOR and (MAPK/ERK) targets due to deficiency of *Fragile X mental retardation protein* (FMRP). These two signaling pathways regulate cellular functions, specifically in the central nervous system (CNS).[45,46,47]

Case report of metformin use in 7 FXS patients consisting of 1 case of type 2 DM, 3 cases of PWP, two adults with obesity and/or behavioral problems, and one child with FXS. Metformin is effective for obesity in Prader-Willi Phenotype (PWP) sufferers in FXS. After 6-12 months of use, metformin produces behavioral improvements in reduced irritability, hyperactive behavior, and social avoidance, and increased social responsiveness. Although individuals with FXS tend to experience cognitive decline over time, especially in verbal communication, administration of metformin also improves conversational language skills.[48]

Elaborate on the inconsistency of results across studies mentioned in the article. Could factors like study design, dosage, or patient population account for these discrepancies? (this part before the future direction. Preclinical studies and off-label clinical use have identified potential additional therapeutic applications for metformin. Off-label use of metformin underpinned its evaluation in gestational diabetes, and there are anecdotal accounts of potential value in several non-diabetic insulin-resistant and/or hyperinsulinaemic conditions. A comprehensive overview of the reported associations between metformin and a wide range of health outcomes, in terms of efficacy and safety, by incorporating evidence from meta-analyses of observational and intervention studies. Extensive preclinical studies and observational clinical data provide encouraging support for further detailed investigation of possible therapeutic repurposing for metformin, particularly considering its much lower cost than current treatments for some conditions.

Despite many studies, evidence on metformin in observational studies generally does not seem very reliable, as can be inferred by substantial heterogeneity between studies, small study

effects, and excess significance. While evidence from randomized trials suggests only a few effects with strong evidence for some benefit, even those may exhibit other caveats. For example, most of the meta-analyses of RCTs did not report information on the follow-up durations and dosage of interventions, making it challenging to evaluate metformin use's time or dose-response effect. In addition, fewer meta-analyses examine adverse effects as the primary outcome. The only adverse effects examined as primary outcomes were the gastrointestinal events.

Although more than half of the meta-analyses of observational studies showed a statistically significant effect of metformin on different cancer outcomes, none of the meta-analyses of RCTs did. However, there was a lower power of meta-analysis of RCTs, justified by a generally lesser number of participants included. In several of the included RCTs, cancer was not the primary outcome but rather an adverse effect.

Observational studies are more prone to bias, which could also justify the discordance in significance between different meta-analyses. The five meta-analyses of observational studies initially stratified as convincing or highly suggestive were downgraded to be weak or even non-significant after restricting the analysis to prospective studies and correcting data anomalies the original systematic reviewers had missed. Reverse causality is a major threat in case-control studies or retrospective cohort studies. The role of reverse causality has been previously suggested in pancreatic and liver cancer since both types of cancer seem to increase the risk of developing diabetes and, therefore, of taking metformin. The fact that different comparison groups have been used in different component individual studies to compare the effects of metformin might have also led to some biases.

In some cases, metformin users were compared to insulin or sulfonylurea users, which are therapies indicated for later stages of the disease. In these cases, the results might be affected by indication bias overestimating the beneficial effects of metformin on cancer outcomes. In some studies, the comparator group was non-diabetic people, which might have underestimated the effects of metformin by ascertainment bias in the diabetic group.

FUTURE DIRECTION

Metformin has significant anti-aging effects and displays a promising perspective in attenuating aging-related diseases. There are several important issues that need to be addressed. First, multicenter, large-scale, double-blind, randomized, placebo-controlled trials are required to further investigate and compare metformin's effects on aging and major aging-related diseases. Second, precision or personalized medicine should be considered when using metformin for aging-related diseases. The type of responder or non-responder should be addressed. The patient's genetic background, the dose of medicine, and the approach of administration, in particular, should be taken into account, and then a personalized treatment strategy should be developed.

Besides that, due to limitations of the administration of metformin in the clinics because of its short half-life and relatively low bioavailability, the design of novel drug delivery systems to

improve drug bioavailability, enhance drug stability, and reduce side effects of metformin is largely appreciated in clinical applications. To establish the molecular mechanisms and pathways of aging, it is important to investigate potential hormone-metformin interactions in male and female animals of varying ages, as the age of starting metformin treatment determines whether an increase in mean and maximum life span occurs. At last, a better understanding of distinct molecular mechanisms through which metformin attenuates different aging-related diseases is still poorly understood and remains to be investigated in great detail so it can facilitate the development of novel and efficient strategies for the prevention and treatment of aging-related diseases by metformin.

CONCLUSION

Considering the ethical and practical implications of prescribing metformin off-label for anti-aging in broader, non-clinical populations, the conclusion is still not clear

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Conflict of Interest

The authors declare no competing interest.

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