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A reappraisal on metformin

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ABSTRACT

This review investigates the different biological effect of Metformin (MET) in different conditions. MET is an oral antidiabetic drug used for the treatment of type 2 diabetes mellitus (T2DM) particularly in overweight people. The main mechanism of action of the MET is inhibition of hepatic glucose production and reduction of insulin resistance. In addition to its antidiabetic effects, MET is also found to be related with the risk for development of several human solid cancers types such as colorectal, breast and pancreas cancer in the diabetic patients. Nowadays according to some researches, MET is believed to decrease or prevent aging and mortality. Moreover, clinical and experimental evidence has shown that MET has beneficial effects in patient with obesity, polycystic ovarian syndrome and Alzheimer's disease. Recent studies have shown that activation of adenosine monophosphate-activated protein kinase (AMPK) by MET can explain its beneficial metabolic effects. In this manuscript, a reevaluation of mechanisms as well as pharmacokinetic properties, genetic variants of transporters, drug-drug interactions, side effects and potential clinical benefits of MET have been reviewed.

1. Introduction

Diabetes mellitus (DM) is the most widespread metabolic disease and it becomes a heavy burden of public health systems (Ke et al., 2016). According to World Health Organization (WHO) Global report on diabetes in 2016, the number of people with diabetes has increased from 108 million in 1980 to 422 million in 2014 (World Health Organization Grod, 2017). International Diabetes Federation (IDF) says that 1 in 11 adults have diabetes in the world and the number of people with diabetes is estimated that there will be 642 million in 2040 (IDF Diabetes Atlas, 2017). Especially it has seen to diabetes prevalence has risen more rapidly low- and middle-income countries, compared to in high-income countries (World Health Organization Grod, 2017).

Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are the types of DM. T1DM, insulin-dependent diabetes mellitus, occurs when the pancreas fails to produce enough insulin for glucose metabolism. T2DM, non-insulin-dependent diabetes mellitus, is a chronic condition characterized by increased blood glucose levels as a result of resistance to the action of insulin. It begins with insulin resistance, a condition in which cells fail to respond to insulin properly (WHO Diabetes Fact Sheet RN, 2017). T2DM can lead to numerous micro and/or macrovascular complications and may cause substantial disability (Charokopou et al., 2015). As long as the disease progresses, a lack of insulin may also develop (Chandalia and Das, 2012). Deteriorations of beta cell function and insulin resistance are two fundamental pathophysiologic defects of T2DM. Recent studies suggest that beta cell dysfunction develops before onset of T2DM (Saisho, 2015). It was noticed that greater glycemic variability and poorer glycemic control because of β-cell dysfunction may result in increased risk of diabetic disorders. It has been proven that at the time when T2DM was established, the loss of beta cell function was shown to reduce by 50% and this decline of beta cell function progressed over time, although traditional antihyperglycemic therapy had been applied (Wajchenberg, 2007). In order to postpone the progress of disease, new therapies are required to persistently act on beta cell failure and insulin resistance (Ke et al., 2016).

The medical history of metformin (MET) goes back to the use of *Galega officinalis* (the French lilac) extracts, which was utilized in Chinese medicine and also in medieval Europe to treat halitosis and polyuria (Bailey and Day, 1989; Witters, 2001). Later, in France, it was also described that MET is used to treat symptoms of diabetes until the early 1930s (Parturier and Hugnot, 1935). According to researchers in the late 1800s, *Galega officinalis* was rich in guanidine, which had hypoglycemic properties in animals. Thus, the anti-diabetic action of plants was explained (Watanabe, 1918). However, galegine, an isoprenyl derivative, was used in the treatment of diabetes in humans in the 1920s due to its fewer side effects compared to guanidine, whereas the clinical usage of guanidine was determined to be toxic (Muller and Rheinwein, 1927). In the same period, MET that is dimethyl biguanide was also synthesized and has strong effects on lowering blood glucose

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levels *in vivo* (Hesse and Taubmann, 1929). However, since insulin was discovered during the same decade, its clinical application for treatment of diabetes was hindered. In addition to MET, the more potent biguanide derivatives called as phenformin and buformin used clinically to treat T2DM (Bailey and Day, 2004). In the 1950s, Jean Sterne a physician at the Hospital Laennec and Aron Laboratories in Paris independently investigated biguanides as antidiabetic agents and determined MET was the best option for clinical development called "Glucophage" (glucose eater) (Fischer and Ganellin, 2010). Initially, the latter drugs were more widely used; yet, in the 1970s, phenformin and buformin were correlated with life-threatening lactic acidosis (Nattrass and Alberti, 1978). In 1994, MET was approved for use in the United States. The American Diabetes Association and the European Association for the Study of Diabetes have suggested it as the first line oral treatment for T2DM since 2009 (Thomas and Gregg, 2017).

MET is the most widely used an oral antihyperglycemic drug for the treatment of T2DM today. It has also other beneficial effects beyond glycemic control, especially on several disorders such as cancer, aging, Alzheimer's disease, polycystic ovarian syndrome, and obesity due to its different biological properties (Maniar et al., 2017). Nowadays, in addition to its use in diabetes, it is being searched for its role on these subjects.

The aim of this review is to reevaluate the mechanisms and pharmacokinetic properties, genetic variants of transporters, drug-drug interactions, side effects, and give more detailed information about potential clinical benefits of MET in terms of aforementioned major diseases.

2. Methods

2.1. Strategy, inclusion and exclusion criteria of the review

During the literature review, key words and index/subject terms related to the topic were searched in at least publication titles, article titles, article abstracts, and author names. In this literature review, all important publications were taken into consideration by utilizing the peer-reviewed journals, non-peer reviewed literature, and expert reports and examinations. In the context of the review, a total of 120 source references covering the main characteristics and the various clinical impacts, benefits, and outcomes on specific disorders such as cancer, neurology, endocrine, metabolism, and aging of MET was included in the study. While there was no specific exclusion criterion in the study, mostly the publications of 2005 and later were considered.

3. Physicochemical and pharmacokinetic properties of metformin

MET has been synthesized in 1922, and involves the reaction of dimethylamine hydrochloride (1) and 2-cyanoguanidine (2) (Werner and Bell, 1922). (Fig. 1).

The acid dissociation constant values (pKa) of MET is 11.5. Due to its alkaline characteristic, the absorption of MET is higher in alkaline environment. As a consequence its high solubility and low permeability, MET is a class III drug according to the Biopharmaceutics Classification System (BCS) (Kim et al., 2014). Because of the low lipophilicity, MET cannot pass through the cell membranes rapidly by passive diffusion. In the present, there are different bioavailability studies that researchers aim to invent more lipophilic derivatives of MET for better oral absorption and bioavailability (Graham et al., 2011).

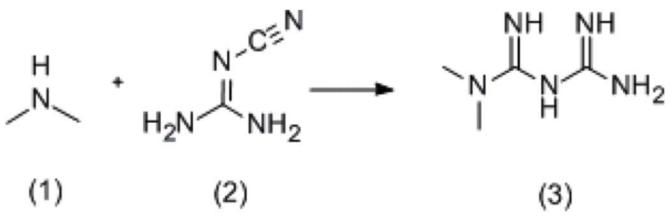

Fig. 1. Chemical synthesis of metformin (Werner and Bell, 1922).

MET is absorbed dominantly from the small intestine (Graham et al., 2011), but this absorption is slow and it is not entirely (Stage et al., 2015; Holguín et al., 2011). The oral bioavailability of 0.5–1.5 g MET is 50–60% (Holguín et al., 2011) and its maximum plasma concentration occurs 2–3 h after dosage (Stage et al., 2015). The half-life of MET is 6.2 h and effects of MET continues for 8–12 h (Drugbank, 2016). MET is rapidly distributed following absorption and it does not bind to plasma proteins. It is not metabolized in the liver and it is excreted without changing in the urine without metabolic change (Graham et al., 2011). It is filtrated freely by glomerular secretion (Stage et al., 2015). The population mean for renal clearance (CLR) is 510 ± 130 ml/min and it is also secreted in the proximal tubules (Graham et al., 2011).

The principal route of MET elimination is active tubular secretion in the kidney. MET is widely distributed into body tissues including intestine, liver, and kidney by organic cation transporters (Graham et al., 2011). These transporters which play a role in the transport of MET, are organic cation transporters (OCTs), multidrug and toxin extrusion transporters (MATEs), and plasma membrane monoamine transporter (PMAT) (Stage et al., 2015).

The intestinal absorption of MET may be primarily mediated by PMAT, which is encoded by gene SLC29A4, and expressed on the luminal side of enterocytes (Zhou et al., 2007). Currently there are no in-vivo data regarding the role of PMAT in the disposition and pharmacological effect of MET. OCT3 (gene SLC22A3) is also expressed on the brush border of the enterocytes and it may contribute to MET uptake (Graham et al., 2011; Muller et al., 2005). In addition, OCT1 (gene SLC22A1), which is expressed on the basolateral membrane and cytoplasm of the enterocytes, may ease the transfer of MET into the interstitial fluid (Muller et al., 2005). The hepatic uptake of MET is mediated primarily by OCT1 and possibly by OCT3. Both of these transporters are expressed on the basolateral membrane of hepatocytes (Graham et al., 2011; Takane et al., 2008; Chen et al., 2010; Nies et al., 2009). In OCT1-deficient mice, the hepatic MET concentration in the liver was significantly lower when compared to the control mice. As a result, it is realized that OCT1 is essential for the hepatic uptake of MET (Shu et al., 2007). Moreover, the glucose-lowering effects of MET were completely prohibited in the OCT1-deficient mice. Also, MET is a good substrate for human multidrug and toxin extrusion 1, MATE1 (encoded by the gene SLC47A1) and MATE2-K (gene SLC47A2) (Takane et al., 2008; Tsuda et al., 2009a; Sato et al., 2008; Tanihara et al., 2007). MATE1 is highly revealing, in the liver, kidney, and skeletal muscle (Otsuka et al., 2005), and may contribute toward the excretion of MET from both the liver and the kidney. However, the role of MATE1 in hepatic secretion has been questioned, as biliary excretion of MET seems to be insignificant in humans (Graham et al., 2011). Data from a mouse study about MATE1 suggest that, at least in rodents, biliary excretion of MET occurs (Ito et al., 2010). The uptake of MET from circulation into renal epithelial cells is primarily expedited by OCT2 (gene SLC22A2) (Takane et al., 2008), which is expressed predominantly at the basolateral membrane in the renal tubules. Renal excretion of MET from the tubule cell to the lumen is mediated through MATE1 and MATE2-K (Tsuda et al., 2009a, 2009b; Sato et al., 2008; Ito et al., 2012). MATE1 and MATE2-K are expressed in the apical membrane of the renal proximal tubule cells, and studies in healthy individuals suggest that they contribute to the renal excretion of MET (Kusuhara et al., 2011). Furthermore, P-gp (gene ABCB1) and BCRP (gene ABCG2) are involved in the efflux of metformin across placental apical membranes (Hemauer et al., 2010).

4. Mode of action of metformin

The mode of action of MET is different from other classes of oral antihyperglycemic agents (Sanders et al., 2007). The ability of MET to

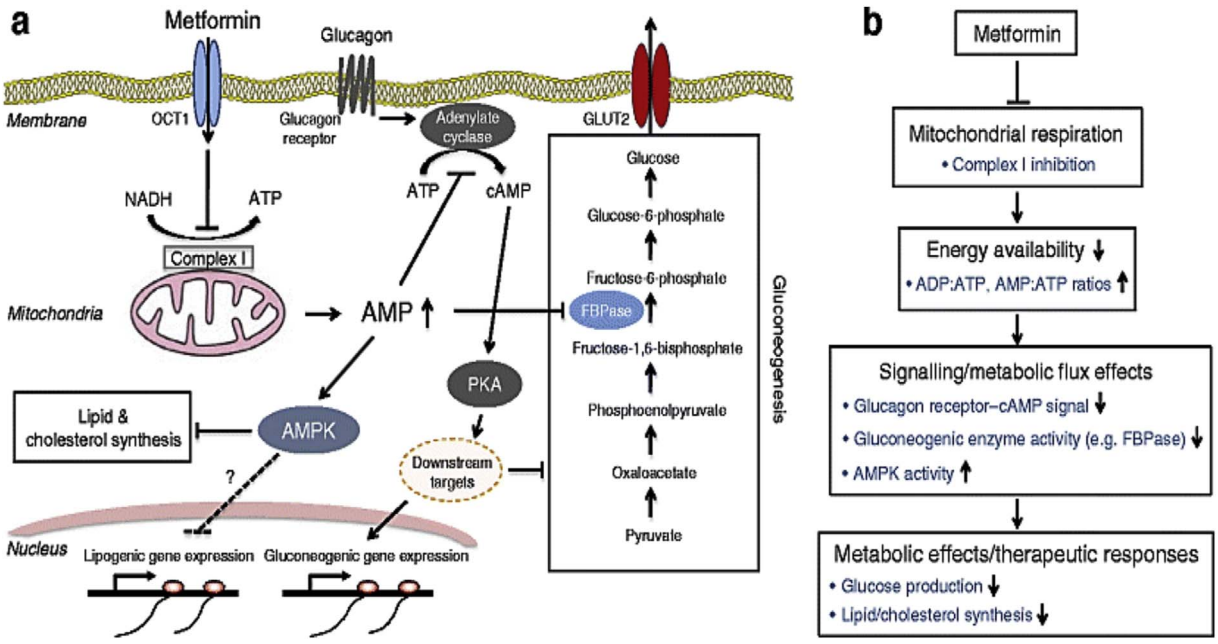

Fig. 2. The molecular mechanisms of metformin on the liver cell (Rena et al., 2013).

suppress both hepatic gluconeogenesis and other anabolic pathways such as lipid and cholesterol biosynthesis is partly related to transient inhibition of the mitochondrial respiratory chain complex I and indirect activation of the energy-sensing AMP-activated protein kinase (AMPK) pathway (Shaw et al., 2005; Viollet et al., 2012; Maziere et al., 1988). The primary molecular target of MET is believed to be mitochondria, where it reduces complex I of the electron transport chain, resulting in a reduction in oxidative phosphorylation and ultimately a reduction in the synthesis of ATP (Sanders et al., 2007).

Increased AMP binds to the AMPK binding domain and causes allosteric conformational change, as a result of this, it activates the catalytic domain of AMPK (Sanders et al., 2007). Apart from activates AMPK, increased AMP inhibits cAMP–PKA and fructose-1,6-bisphosphatase (FBPase). This mechanism leads to inhibition of gluconeogenesis (Rena et al., 2013) (Fig. 2). While the ratio of AMP/ATP increases, MET has been shown to activate AMPK through an upstream kinase, LKB1 (Shaw et al., 2005). By the activation of AMPK, the cell is derived from an anabolic to a catabolic state. However, there is a still controversial hypothesis about whether MET activates AMPK directly by altering the cell's energy status, as well as AMP/ATP ratio, or by a LKB1 mediated process (Algire et al., 2011; Dowling et al., 2011; Jalving et al., 2010; Pierotti et al., 2013).

Through activating AMPK, gluconeogenesis genes in the liver and genes encoded glucose transporters in muscle cells (e.g. GLUT1) are transcriptionally regulated by MET. Thus, MET inhibits gluconeogenesis, and induces glucose uptake into muscle cells and lowers blood glucose and insulin level in T2DM (Dowling et al., 2011). He et al., in 2009 showed that MET works in different way from insulin to maintain the glucose hemostasis in T2DM (He et al., 2009). MET might also affect the host metabolism through reduction in hepatic gluconeogenesis indirectly, leading to reduced circulating insulin levels and decreased insulin/IGF-1 receptor-mediated activation of the PI3K pathway (Leone et al., 2014). AMPK blocks signaling via the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) pathways, downstream of the insulin and IGF1 receptors (Sen et al., 2014; Pulito et al., 2013; Malek et al., 2013). Some recent clinical trials did not show favorable effect of IGF-1 antibody or somatostatin analog on anti-cancer outcome despite the reduction of insulin and IGF-I levels (Pollak, 2012a, 2012b; Pritchard et al., 2011). Contrary to popular knowledge, it was reported that metformin was not an insulin sensitiser in muscle or adipocytes in the case of no weight loss, which was common in patients treated with biguanide (Abdul-Ghani and DeFronzo, 2017). In a stable manner, they could not detect any biguanide in the muscle following the intravenous administration of 11C-metformin. Since GLP-1 RAs induce significant weight loss, it also improves insulin susceptibility of muscle. Therefore, GLP-1 RAs, not metformin, ameliorate the two major deficiencies in T2DM patients, i.e., β-cell dysfunction and muscle insulin resistance (Abdul-Ghani and DeFronzo, 2017).

5. Metformin and the gut microbiome

The human gut microbiota contains 10–100 trillion microorganisms (Hur and Lee, 2015). The gut microbiota has an important role in harvesting energy from food. In addition, it has significant influence on metabolic processes, and immune modulation (Lee and Ko, 2014). In recent years, the gut microbiome and the metagenome have been attracting attention. As a result of the researches conducted in this area, the gut microbiome and the metagenome are considered to contribute to progress many diseases, such as T2DM, obesity and the metabolic syndrome (Lee and Ko, 2014; McCreight et al., 2016; Miele et al., 2015). Moreover, it is known that dysbiosis of the gut microbiota is associated with many other diseases, such as autism, cardiovascular disease and inflammatory bowel disease (Lee and Ko, 2014). Therefore, researchers have focused on the modulation of gut microbiota for new therapeutic strategies in these diseases (Miele et al., 2015).According to recent studies, the gut microbiota is considered to have influence on the efficacy of MET (Zhang et al., 2015).

Recently, Shin et al. investigated that the association between antidiabetic effect of MET and alterations of the intestinal microbial composition by using either a normal-chow diet-fed or a high-fat diet-(HFD)-fed mice. In the result, it was found that the HFD-fed mice treated with MET have a higher abundance of *Akkermansia muciniphila* than HFD-fed control mice. Furthermore, when *A. muciniphila is administrated to* HFD-fed mice without MET, it was seen that glucose tolerance, enhanced and attenuated adipose tissue inflammation (Shin et al., 2014). Shin et al. suggested that the modulation of the gut microbiota may contribute to the antidiabetic effects of MET (Zhang et al., 2015; Shin et al., 2014).

In another study conducted by McCreight et al., they demonstrated

that the abundance of *A. muciniphila* decreased in obese and type 2 diabetic mice (Everard et al., 2013).

Lee and Ko investigated the relationship between MET treatment and the gut microbiota by using a mouse model of HFD-induced obesity with and without MET treatment. After MET treatment, serum glucose levels, body weight and total cholesterol levels were observed to improve in the HFD-fed mice. Additionally, *A. muciniphila* and *Clostridium cocleatum* abundances were found to increase significantly in HFD-MET group. Lee and Co also demonstrated the effects of MET on growth of *A. muciniphila* as in vitro (Lee and Ko, 2014).

6. Clinical uses of metformin beyond diabetes

6.1. Metformin and cancer

Recently, MET had a significant impact on the advanced cancer chemoprevention (Kourelis and Siegel, 2012). An initial epidemiological report conducted by Evans et al. (2005) gained the attention of the oncology field when they found that diabetic patients taking MET, when compared to other patients treated with other hypoglycemic therapies, had a significant reduction in cancer risk. These results sparked widespread MET research, ranging from the mechanistic studies to determine its anti-roliferative effect on cancer cells, to clinical trials in non-diabetic patients with various malignancies (Pollak, 2012c; Dowling et al., 2012). An addition to it's benefit for MET use in oncology is that it's known to modulate energy metabolism, which is a topic that is re-emerging in the cancer field. For example, cancer cells are often more metabolically active than surrounding non-malignant tissue. As a consequence of this phenotype, any opposition to glucose utilization by low-energy mimetics such as MET may inhibit tumor proliferation. In fact, recent studies have indicated that tumors carrying mutations in metabolic stress regulators such as LKB1 and p53 undergo substantial apoptosis when treated with biguanides (Algire et al., 2011; Shackelford et al., 2013; Buzzai et al., 2007).

MET dose and time dependently caused ATP reduction, AMP accumulation, increased ratio of AMP to ATP and AMPK activity (Stephenne et al., 2011). Therefore, MET generally is believed to work on cancer cells by activation of AMPK, acting on mitochondrial respiration and leading to an imbalance of energy homeostasis in cancer cells. Interestingly, the study also suggests that MET can directly inhibit mTORC1 signaling by suppressing RAG GTPase protein independent of AMPK activation (Kalender et al., 2010). In human prostate cancer cell lines, MET also induced cell cycle arrest by inhibiting the expression of cyclin D1 and retinoblastoma-protein, two key regulators of the cell cycle, resulting in reduction of cyclin D1 level and eventually G1 cell cycle arrest, independent on AMPK activation (Ben Sahra et al., 2008).

Preoperative treatment with MET did not significantly affect tumor cell proliferations estimated by Ki-67 staining in primary breast cancer tissue, but a different impact on Ki-67 was observed according to different level of insulin resistance with a small decline in Ki-67 in patients who have HOMA index more than 2.8, suggesting high insulin resistance (Bonanni et al., 2012). This situation is consistent with the report that the decrease in hyperglycemia by MET at postprandial state is more than at fasting state and the decrease in hyperinsulinemia is greater if it was present as baseline (Pollak, 2013).

In addition to activation of AMPK and inhibition of mTOR, MET has also been proposed to increase the activity of tumor suppressor p53. AMPK is also involved in p53-mediated cell cycle arrest induced by MET (He et al., 2009; Jones et al., 2005). A recent study discovered the increased oxidative phosphorylation in family members of Li-Fraumeni patient who carries the TP53 mutation, suggesting that p53 regulates mitochondrial respiration and MET may have therapeutic value by reducing oxidative phosphorylation in Li-Fraumeni patients (Wang et al., 2013).

MET reduces chronic inflammatory responses, at least partially by inhibiting the production of tumor necrosis factor alpha, preventing tumor development. In addition, studies have demonstrated that MET reduces production of reactive oxygen species (ROS) through inhibition of mitochondrial complex I, the cellular source of ROS production, to reduce DNA damage and mutagenesis (Li, 2011; Hadad et al., 2011). MET is also reported to inhibit drug resistance, decreases fatty acid synthesis and PGE2 synthesis (Pierotti et al., 2013).

AMPK inhibits the biosynthesis of estrogens and the secretion of leptin, which known to increase cancer cell proliferation and affect energy utilization and stimulates adiponectin secretion, which might inhibit tumor cell growth (Wolin et al., 2010; Wysocki and Wierusz-Wysocka, 2010). In summary, MET most likely works through modulating host environments as well as a target cancer cell to effectively suppress cancer growth.

To enlighten the anticancer effect of MET, there are a lot of researches which have been done recently. Moreover, not only using MET alone, but also using with some chemotherapeutic drugs has been studied. Teixeira et al. (2013) have studied with NC1-H460 human lung cancer cells by treating with antineoplastic drugs (cisplatin and etoposide) and MET in 2013. They have found that the use of MET as monotherapy reduced the metabolic viability of the cell line studied. Combining MET with cisplatin or etoposide produced a synergistic effect and was more effective than was the use of cisplatin or etoposide as monotherapy. In conclusion, MET, due to its independent effects on liver kinase B1, had antiproliferative effects on the NCI-H460 cell line. When MET was combined with cisplatin or etoposide, the cell death rate was even higher. In 2014, there were 4 different studies on the anticancer effect of MET. Firstly, Ali Dastranj Tabrizi et al. (2014) have divided 43 patients into two groups and have treated with 500 mg bid MET and 40 mg daily megestrol. In the first group which is treated with MET, endometrial atrophy has increased 95.5%; however, in the second group, endometrial atrophy increases just 61.9%. Overall, MET could be used as an effective antiestrogenic agent in control of abnormal endometrial proliferative disorders. Secondly, in research which have been done by Soffer et al. (2015), 66,778 women patients with diabetes and used MET and other antidiabetic drugs are followed, and drug categories are MET only; MET + regimens; non-MET regimens; non-users. As a result, MET + regimens group had a 15% lower breast cancer risk when compared to MET only. However, there is no difference in overall cancer risks non-MET users comparing with MET users. Thirdly, Chen-Pin et al. (2014) have followed 71,999 men with T2DM, without prior cancer or liver diseases, nor prescription of thiazolidinediones or insulin between FY2003-FY2013. Cox proportional hazard analyses (adjusting for covariates and propensity scores of MET use) were conducted to compare the hazard ratio (HR) of PCa associated with the MET use between statins or finasteride users and non-users. Therefore, MET was associated with reduced PCa risk in men with T2DM. The effect of MET increases with statins, but it decreases with finasteride on Pca. In a study which has been done by Sun et al. (2014) HNE1/DDP human nasopharyngeal carcinoma (NCP) cells were treated with MET, cisplatin (CIS) and their combination in defined concentrations. In conclusion, low concentrations of DDP had almost no inhibitory effects on cell invasion and migration. Cell invasion and migration decreases significantly when DDP combined with MET. In the present study, with an increasing concentration of MET, the expression of MMP-9 was reduced, whereas there was a significant increase of E-cadherin. Taken together, CIS + MET has effects on proliferation, invasion, and migration of human NPC cells. In a study by Cai et al. (2015) they consist both in vitro and *in vivo*. As in vitro, human esophageal squamous cell carcinoma cell lines, EC109 and EC9706 were treated with, AICAR, MET and Compound C (AMPK Inhibitor). As *in vivo*, animals were randomized into control and experimental group (7–10 mice per group), and they were treated with 250 mg/kg/d MET. At the end of this study, MET inhibits the growth of ESCC cells, both in cell cultures and in an animal model. AMPK, p53, p21CIP1, p27KIP1 and cyclinD1 are involved in the inhibition of tumor growth that is induced by the MET and cell cycle arrest in ESCC. To conclude, MET has

the potential for use in the treatment of Esophageal cancer (ESCC). The latter is case-control analysis done by Becker et al. (2015) from 1995 to 2014 using the UK-based Clinical Practice Research. Cases were diagnosed with thyroid cancer for the first time; six controls per case were matched prior to the index date according to sex, age, general practice, calendar time, and the number of active years. The Cases had a first-time diagnosis of thyroid cancer; six controls per case were matched on age, sex, calendar time, general practice, and the number of years of active history in the database prior to the index date. Odds ratios (ORs) and related 95% confidence intervals (95% CI) adjusted for diabetes mellitus, smoking, and body mass index (BMI) were evaluated. As a result, the relative risk estimate was highest in long-term (≥30 prescriptions) users of MET (adjusted OR 1.83, 95% CI 0.92–3.65), based on a limited number of 26 exposed cases. No such association was found in users of sulfonylurea, insulin, or thiazolidinediones (TZD). Neither diabetes diagnosis, nor diabetes duration altered the risk of thyroid cancer. To conclude, neither use of MET nor of other antidiabetic drugs were associated with a decreased risk of thyroid cancer.

By reading these and more articles about MET's antineoplastic effect, it is realized that there is still a controversy on this topic.

6.2. Metformin and aging

Current treatments for diseases related to ageing "just exchange one disease for another", says physician Nir Barzilai. That is because people treated for one age-related disease often goes on to die from another relatively soon thereafter. "What we want to show is that if we delay ageing, that's the best way to delay disease." (Check Hayden, 2015) Recently, it was announced an ambition project of clinical trial called TAME (Targeting Aging with Metformin) proposed by Nir Barzilai and colleagues (Check Hayden, 2015; Hall, 2015). They are going to give MET during 5–7 years to 3000 people aged 70–80 years who already has one or two of three age-associated diseases (heart disease, cancer, cognitive decline). They will give the drug MET to thousands of people who already have one or two of three conditions—cancer, heart disease or cognitive impairment—or are at risk of them. People with T2DM cannot be enrolled because MET is already used to treat that disease. The participants will then be monitored to see whether the medication forestalls the illnesses they do not already have, as well as diabetes and death. On 24 June, researchers will try to convince FDA officials that if the trial succeeds, they will have proved that a drug can delay ageing. That would set a precedent that ageing is a disorder that can be treated with medicines, and perhaps spur progress and funding for ageing research (Check Hayden, 2015).

From this viewpoint, Bannister et al. have done a research with patients in 2014 (Bannister et al., 2014). Bannister and his colleagues use retrospective observational data from UK Clinical Practice Research Datalink (CPRD) from 2000. The people who have T2DM and use MET or sulphonylureas (SU) as first-line treatment were selected. In this research, 78,241 subjects were treated with MET, 12,222 were treated with SU, and 90,463 were non-diabetic people. As findings, there were 7498 deaths in total, representing unadjusted mortality rates of 14.4 and 15.2, and 50.9 and 28.7 deaths per 1000 person-years for metformin monotherapy and their matched controls, and sulphonylurea monotherapy and their matched controls, respectively. As a conclusion of this study, patients with T2DM initiated with MET monotherapy had a longer survival than did matched, non-diabetic controls. On the other hand, those treated with SU had markedly reduced survival comparing with both matched controls and those receiving MET monotherapy.

6.3. Alzheimer's disease and metformin

Alzheimer's disease (AD), which is a neurodegenerative disease, is the most common type of dementia. Although the etiology and pathogenesis of AD are not exactly understood, it is considered that AD process is associated with the extracellular accumulation of amyloid-β (Aβ) protein in neuritic plaques, the hyperphosphorylation of tau protein to form neurofibrillary tangles within hippocampal and cortical neurons, neuron loss, synapse loss, and brain atrophy. Researches show that diabetes, stroke, atherosclerosis, obesity, a high-fat diet, metabolic syndrome and oxidative stress, increase the risk of AD (Asadbegi et al., 2016). Besides, insulin resistance has harmful effects on neuronal development, synaptic plasticity, behavior and cognition (Maniar et al., 2017). It has been found that T2DM increases the risk of developing AD (Moore et al., 2013; Chen et al., 2009).

Recently, MET is considered as a promising drug for AD. In a research which is published in "Proceedings of the National Academy of Sciences of the United States of America" (PNAS), Kickstein et al. have reported that MET reduced tau phosphorylation in murine primary neurons in vitro and *in vivo*. MET inhibited protein phosphatase 2A activity (PP2A) rather than mTOR, via the regulatory subunit of PP2A-α4 and the ubiquitin ligase MID1. However, MET did not show a considerable effect on the phosphorylation of the AMPK. In accordance with these results, it has been thought that long-term use of MET may be useful for prophylaxis and/or therapy of AD (Kickstein et al., 2010).

In a study by Gupta et al., they investigated the effect of the MET on neuronal insulin resistance and AD-associated neuropathological alterations in an in vitro neuronal insulin-resistant model. In conclusion, they firstly found a direct association between chronic hyperinsulinemia and AD. Secondly, they observed that MET restored neuronal insulin resistance and prevented AD-associated pathological changes (Gupta et al., 2011).

Wang et al. have demonstrated that MET enhanced neurogenesis and spatial memory formation in the culture of both human and rodent neurons by activating the atypical PKC-CBP pathway, which is significant for neural precursor differentiation (Wang et al., 2012).

In another study by Chen et al. in db/db mice, they found that MET improved memory impairment, inhibited neuronal apoptosis and Aβ accumulation in the hippocampus as well. They also observed that MET noticeably influenced on RAGE-mediated transport of Aβ across the blood–brain barrier, it did not have an important effect on LRP1-mediated transport of Aβ (Chen et al., 2016).

However, there are contradicting studies regarding the beneficial effects of MET for AD. In 2009, Chen et al. demonstrated that MET increases the generation of Aβ peptides by activating AMPK. Because the aggregation of Aβ peptides is one of the pathogenesis of AD, they concluded that MET may trigger the development of AD (Chen et al., 2009).

In another case-control study including 14,172 participants 65 years of age or older, the authors reported that long term use of MET was associated with increased risk of developing AD, compared to nonuse of it (Imfeld et al., 2012).

In a study conducted by Moore et al., it was demonstrated that patients with using MET had worse cognitive performance compared to control group. Because it is possible to MET impair absorption of vitamin B12, it is considered that MET-induced vitamin B12 deficiency may cause neurodegenerative diseases such as AD (Moore et al., 2013).

6.4. Polycystic ovarian syndrome and metformin

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women (Naderpoor et al., 2015) and it affects 5%–10% of women of fertility age (Tosca et al., 2011). It is characterized by reproductive features such as infertility, miscarriage, pregnancy complications (Naderpoor et al., 2015), anovulation, androgen excess, polycystic ovaries, raised luteinizing hormone (LH) levels, increased LH: follicle-stimulating hormone (FSH) ratio (Tosca et al., 2011), and metabolic features such as obesity, insulin resistance, prediabetes, T2DM and cardiometabolic. Insulin resistance and hyperandrogenism are the most important factors in the pathophysiology of PCOS. Hyperinsulinemia brings about to increase in androgen production (Naderpoor et al., 2015; Palomba et al., 2009) and reduce in sex

hormone-binding globulin (SHBG). This mechanism leads to hyperandrogenism (Naderpoor et al., 2015; Patel and Shah, 2017).

Besides lifestyle modification which is the first line treatment; the oral contraceptive pill, MET, cyclic progestins, anti-androgens and fertility treatments are used in PCOS (Naderpoor et al., 2015). MET has been used for PCOS treatment because of its insulin-sensitizing effect (Tosca et al., 2011; Patel and Shah, 2017).

Although the efficacy of MET in PCOS is debatable, it has been found to regulate menstrual cycle, ovulation frequency and clinical symptoms of hyperandrogenism (Patel and Shah, 2017). However, some researchers have found that OCs are much more effective in terms of in regulating menstrual cycles than MET (Palomba et al., 2009). MET takes effect in PCOS by reducing insulin resistance and inhibiting ovarian androgen production (Thomas and Gregg, 2017; Naderpoor et al., 2015; Patel and Shah, 2017), through steroidogenic acute regulatory protein and 17alphahydroxylase (Naderpoor et al., 2015). MET also has effect on modulate LH secretion by decreasing LH pulse amplitude. The efficacy of MET in PCOS may be changed depending on MET's dosage and formulation types such as extended-release and immediate-release (Palomba et al., 2009).

A study carried out by Velazquez et al., in 1994 including 26 obese PCOS patients, has found that MET reduced circulating androgen levels and body weight in addition to induce regular menstrual and ovulatory cycles (Palomba et al., 2009).

In the systematic review and meta-analysis by Naderpoor and colleagues, it was compared the effect of lifestyle modification + MET with lifestyle modification ± placebo, and MET alone with lifestyle modification ± placebo in PCOS. As a result of this analysis, including 2372 identified studies and 12 RCTs, it was seen that lifestyle + MET was linked with lower Body Mass Index (BMI) and subcutaneous adipose tissue, and increased number of menstrual cycles compared to lifestyle + placebo over 6 months. In addition, it was shown that MET alone and lifestyle had similar effects on BMI, however, testosterone levels was determined lower with MET alone (Naderpoor et al., 2015).

Another meta-analysis of randomized controlled trials (RCTs) published in 2017, having as its aim to evaluate the efficacy of MET in PCOS reported that MET reduces body mass index (BMI) and waist-to-hip ratio (WHR) as well as improves systolic blood pressure, diastolic blood pressure, triglyceride levels, glucose insulin ratio, and serum testosterone levels as compared with placebo. On the other hand, MET has been observed to not improve metabolism and endocrine outcomes (Patel and Shah, 2017).

6.5. Obesity and metformin

At the present time, obesity is accepted as one of the important health problem in the world because of triggering many disorders such as cardiovascular disease, diabetes, stroke, osteoarthritis and cancer (Maniar et al., 2017). According to 2013 data of The World Health Organization (WHO), 500 million people suffer from overweight and/or obesity (Smieszek et al., 2015). Therefore, treatment of obesity becomes important and it is considered that MET promotes weight loss and it may be beneficial in obesity treatment (Maniar et al., 2017; Smieszek et al., 2015), because of its several actions on adipose tissue. As a result of a randomized study, it was reported that MET may be much more effective as a weight loss agent in children and adolescents compared to adults (Smieszek et al., 2015).

MET has been found effective to decrease adiposity and obesity-associated conditions in both human and animal (Smieszek et al., 2015). It decreases the triglyceride stores by increasing lipolysis and β-oxidation in adipose tissue. It inhibits the mitochondrial complex I and so the energy activating AMPK releases. The activation of AMPK causes to inhibit lipolysis and induce apoptosis. In addition, MET induces increase in mitochondrial mass like leptin, which is a hormone increasing energy expenditure and suppressing appetite. MET also have been shown to increase uncoupling protein 2 (UCP2) levels in white adipose tissue of C57BL/6 mice (Anedda et al., 2008). Recently, Śmieszek et al. investigated the influence of MET on the morphology and proliferation potential of adipose-derived mesenchymal multipotent stromal cells (ASCs) and adipocytes. They observed that MET exhibited an inhibitory effect on the proliferative potential of ASCs in dose- and time-dependent manner in vitro. In this study, MET also affected the circulating concentration of osteopontin (OPN) (Smieszek et al., 2015).

Reducing the absorption of carbohydrates from the gut, suppressing hepatic glycogenesis and hepatic glucose-6 phosphate, and inhibiting hepatic gluconeogenesis are other actions of MET that may be beneficial for obesity treatment (Maniar et al., 2017; Seifarth et al., 2013), so that less glucose are ensured to the adipose tissue for energy storage (Seifarth et al., 2013).

According to many studies, there is a strong link between obesity and insulin resistance. Because MET increases insulin sensitivity, this mechanism produces potential treatment for weight loss (Seifarth et al., 2013; Igel et al., 2016). Additionally, the other mechanisms of MET that is considered useful for treatment of obesity are decreasing in appetite, decreasing of leptin levels both in adipose tissue and serum levels and increasing GLP-1 levels (Seifarth et al., 2013).

The Diabetes Prevention Program Outcome Study (DPPOS) examined the long-term effects of MET on weight loss and waist circumference for 10 years. The MET group was observed to lose body weight 2.06 ± 5.65% and waist circumference 2.13 ± 7.06 cm. However, a placebo group lost weight 0.02 ± 5.52% and waist circumference compared to control group was 0.79 ± 6.54 cm (Long-term safety, 2012).

In a 6-month study, including 199 subjects, the efficacy of MET (up to 2500 mg daily) in obese and overweight patients was investigated. 154 patients (BMI ≥ 27 kg/m2) of 199 subjects were being treated with MET, whereas the other 45 patients did not use MET. Any patients did not change their lifestyle (diet and physical exercise) during the study. In conclusion, it is observed that the weight loss in MET-used patients was considerably greater than the control group. While the patient with use of MET lost weight average 5.8 ± 7.0 kg, the control group gained weight average 0.8 ± 3.5 kg. 47.4% of the 154 MET-used patients lost weight at least 5% and 16.2% of them lost weight at least 10%. In addition, the patients with insulin resistant lost more weight than patients with insulin sensitive (Seifarth et al., 2013).

In 108 patients, the efficacy of acarbose and MET on weight loss and HbA1C level was compared. Initially, the HbA1c of these patients was between 7% and 10% and BMI was greater than 24 kg/m (World Health Organization Grod, 2017). At the end of 24 weeks, it was observed that both acarbose and MET reduced BMI and this reduction was similar (acarbose: 3.3 ± 3.7 vs. MET: 2.7 ± 3.2; $P > .05$). Besides, the reduction in HbA1c level was found to similar between the two groups as well. Whereas 64.7% of the patients using acarbose reached endpoint HbA1c < 6.5%, 57.7% of the patients using MET reached this endpoint (Sun et al., 2016).

7. Genetic variants of transporters and response to metformin

OCTs play a key role in the hepatic and renal transport of MET. OCT1 mediates its hepatic uptake, whereas OCT2 and MATE1 facilitate its renal secretion (Manolopoulos and Ragia, 2014). Genetic variants of OCTs in patients may change absorption, distribution and elimination of MET (Kim et al., 2014; Graham et al., 2011). Studies have demonstrated that genetic polymorphisms in OCTs are responsible for altering its pharmacokinetics and pharmacodynamics (Thomas and Gregg, 2017; Yoon et al., 2013). In the other words, genetic variations on the OCTs may lead to change response to MET in interindividual (Pawlyk et al., 2014).

It has been observed that several genetic variants of OCT1 such as Arg61Cys, Gly401Ser, Met420del and Gly465Arg exhibit damaged transport of MET, in vitro. However, it is vague whether these variants have an effect on the pharmacokinetics *in vivo* or they alter clinical

response. The frequency of these variants may be different in individuals. For example, whereas the frequency of Met420del is 18.5% in Caucasian, it is 2.9% in African Americans and lesser in Japanese and Koreans (Holguín et al., 2011).

In 2009, Tzvetkov et al. investigated the genetic variation relationship (OCT1, OCT2, OCT3, OCTN1 and MATE1) between pharmacokinetics of MET in 103 healthy male Caucasians. Consequently, they found that OCT1 alleles (Arg61Cys, Gly401Ser, 420del, or Gly465Arg) cause to increase renal clearance and decrease hepatic uptake of MET (Tzvetkov et al., 2009).

In another study by Becker et al., in 2009, the association of genetic variation in OCT1 (SLC22A1) with alteration in HbA1c level was analyzed in 102 metformin users. In conclusion, they found that there is substantially association between rs622342$A > C$ polymorphism and HbA1c reduction. Each minor C allele at rs622342 was observed to cause 0.28% less decrease in HbA1c levels (Becker et al., 2009).

Furthermore, apart from the effect of OCT1 variants on MET pharmacokinetics and clinical response, in a study, the influence of seven polymorphisms in OCT1, OCT2, and MATE1 genes on the side effects of MET was evaluated in 246 T2DM patients in MET treatment, of whom 53 experienced gastrointestinal side effects. As a result of this study, it was found that there is a link between two genetic variations in OCT1 (rs628031 and rs36056065) and MET intolerance (Tarasova et al., 2012).

Briefly, numerous studies have demonstrated that polymorphisms in OCTs affect pharmacokinetics and therapeutic response of MET.

8. Drug-drug interactions

Because MET is not metabolized in the liver, the inhibition of OCTs is important in terms of drug–drug interactions (DDIs) (Stage et al., 2015; Ding et al., 2014). Whereas the inhibition of OCT1 which regulates metformin's hepatic uptake causes reduced effect of MET, the inhibition of OCT2 which regulates metformin's renal uptake most likely causes increase systemic disposition of MET due to reduced renal clearance (Ding et al., 2014).

T2DM patients have many disorders apart from T2DM. So other drugs may be needed for treatment of these disorders (Kim et al., 2014). In this situation, it is necessary to pay attention when MET and inhibitors of OCTs are used together (Ding et al., 2014).

Gastrointestinal problems are very common in patients with T2DM. Proton pump inhibitors (PPIs) are used for treatment of gastroesophageal reflux disease. When PPIs and MET are administered together, PPIs may affect the plasma concentration of MET (Kim et al., 2014). In a study by Nies et al., in 2011, they observed that PPIs (omeprazole, pantoprazole, lansoprazole, rabeprazole, and tenatoprazole) substantially inhibited transport of OCT-mediated MET in vitro, in a concentration-dependent manner (Nies et al., 2011).

Ding et al. have reported that the modest pharmacokinetic drug interaction between lansoprazole and MET in a study with 20 healthy male volunteers who received MET co-administered with placebo or with lansoprazole. In this study, it was observed that lansoprazole increased plasma concentration of MET and $AUC_{0\text{-}24}$ by 15 and 17% respectively, and decreased its renal clearance by 13%. In addition, the MET elimination half-life was prolonged from 3.9 to 4.5 h by lansoprazole. In conclusion, Ding et al. has recommended to monitor when MET and lansoprazole are used together long-term in case the risk for MET accumulation, particularly in the patient who has a lot of developing lactic acidosis (Ding et al., 2014).

In a study by Kim et al., they aimed to observe the influence of pantoprazole and rabeprazole on the MET pharmacokinetics in 24 participants. As a result of this study, they found that AUC of MET was 15% and 16%; Cmax of MET was also 15% and 22% greater when it was administered with pantoprazole and rabeprazole, respectively. However, they did not observe a significant difference in the maximum glucose concentration. Although previous searches have indicated that PPIs may also inhibit MATE and OCT2 transporters, in this study, the inhibition of OCT2 and MATE1 by PPIs was considered to be insignificant. On the other hand, the increase in plasma MET levels was thought to be associated with increasing gastric pH by PPIs, because MET dissolves higher in alkaline environment (Kim et al., 2014).

As a result of the search by Stage and co-workers on PubMed, Medline and Embase, although there are a great number of DDI studies regarding MET, they found that some drugs (cimetidine, contrast agents, dolutegravir, phenprocoumon, pyrimethamine, ranolazine, rifampicin, St John's wort, trimethoprim, vandetanib and verapamil) are clinically significant. They also, emphasized that the patients with reduced kidney function were more susceptible to DDIs, because MET is excreted unchanged through the kidney (Stage et al., 2015).

9. Side effects of metformin

The most frequent side effects of MET are gastrointestinal disturbances (Pawlyk et al., 2014; Day and Bailey, 2007), such as diarrhea, nausea, vomiting, flatulence, abdominal pain and loss of appetite (Drugscom, 2016). Gastro-intestinal side effects may tolerate with time. In order to reduce them, the drug can be taken with meals. In addition, initially the drug should be taken low dose and titrated up the dose slowly (Pawlyk et al., 2014). Insufficient nourishment, alcohol intake and co-administration with anti-diabetic drugs such as insulin, sulfonylureas and meglitinides may bring about to hypoglycemia (Igel et al., 2016).

Lactic acidosis is the rare side effect associated with metformin and generally it occurs when there is renal insufficiency. Hence, metformin is contraindicated in patients who have substantial renal dysfunction. Anemia which results from vitamin B12 malabsorption and deficiency has been reported, but it is rare (Pawlyk et al., 2014; Day and Bailey, 2007). Also hypoglycemia can be seen when metformin is used with insulin-releasing oral antidiabetic drugs or insulin (Day and Bailey, 2007).

10. Conclusion

MET is a widely used oral anti-diabetic drug for the treatment of T2DM without causing weight gain over 50 years. Besides MET is mainly used for treatment of DM, it has also anticancer effect by inhibiting cancer cell proliferation and tumor growth. Because activation of AMPK has been considered to may be useful in therapy of cancer, as an AMPK activator is MET can reduce the tumor size. Additionally, decreasing adiposity, activation of AMPK, reducing absorption of carbohydrates, suppressing hepatic glycogenesis and inhibiting hepatic gluconeogenesis by MET are important roles in obesity treatment. Recently, it has also been reported that MET can reduce aging or prevent aging. Although there are controversial conclusions, it has been shown by some researchers that MET may be effective in Alzheimer's disease and significantly reduce dementia. According to recent research results, it is thought to be possible, but not certain, that MET increases SHBG, decreases glucose and free serum testosterone levels, regulates ovulatory menstrual cycles and increases pregnancy rates in patients with PCOS.

This review provides a general introduction, and a comprehensive summary of the benefits, effects and consequences of MET in terms of major diseases such as T2DM, obesity, aging, Alzheimer's disease, polycystic over syndrome, and cancer. The study also highlights important details about the clinical uses of MET beyond diabetes. In the future, researches with larger sample size or meta-analysis studies are needed in order to verify the important and still uncertain questions mentioned here.

Transparency document

Transparency document related to this article can be found online at

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