Mechanisms of Action of Metformin in Type 2 Diabetes and Associated Complications: An Overview

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Abstract: Type 2 diabetes is a major health problem associated with excess mortality and morbidity. Vascular complications are one of the most serious consequences of this disorder. Moreover, type 2 diabetes is also a risk factor for cerebral complications, including cognitive impairment and dementia. However, it has been shown that tight glycemic control contributes to reduce the incidence of diabetes-associated complications. Metformin is a potent antihyperglycemic agent widely used in the management of type 2 diabetes whose main actions are the suppression of gluconeogenesis and the improvement of glucose uptake and insulin sensitivity. This review is mainly devoted to describe the mechanisms of action underlying the antidiabetic effects of metformin. Furthermore, we will present evidence for the protective effects of metformin against diabetes-associated complications mainly cerebral and vascular complications. Finally, we will describe the few known side effects associated to this antidiabetic agent.

Key Words: AMP-activated protein kinase, brain protection, diabetes-associated complications, hyperglycemia, insulin resistance, metformin, type 2 diabetes, vasculoprotection.

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INTRODUCTION

Type 2 diabetes is a polygenic disorder [1] that results from the interaction between genetic predisposition and environmental factors [2]. It is characterized by chronic hyperglycemia, which is a consequence from defects in both insulin sensitivity and β-cells function [3-5]. Indeed, most patients with type 2 diabetes manifest insulin resistance and reduced insulin response to glucose [6]. In 2007, it has been estimated that type 2 diabetes affected approximately 200 million people around the world [7]. Furthermore, the incidence of this disorder is rising to epidemic proportions [8,9].

Type 2 diabetes promotes the development of serious complications that are major causes of morbidity and mortality [10,11]. In fact, this chronic disease is associated with both macrovascular complications, such as myocardial infarction and stroke, and microvascular complications, such as diabetic nephropathy, retinopathy and neuropathy [12]. Moreover, the long-term consequences of diabetes include end-organ damage (kidneys, eyes, heart and nervous system) [13]. Multiple biochemical mechanisms have been proposed to explain the progression of these diabetes-associated complications, including hyperglycemia-induced enhanced polyol activity, increased formation and accumulation of advanced glycation end products (AGEs), activation of protein kinase C (PKC) and increased hexosamine pathway flux [14]. However, Brownlee suggested that hyperglycemia induces an overproduction of superoxide by the mitochondrial electron-transport chain indicating that activation of the oxidative stress by hyperglycemia is a critical factor in the pathogenesis of diabetic complications [15].

Therefore, maintaining normal plasma glucose levels is a key factor to delay or prevent the development of diabetes complications [16,17]. Currently, a diversity of drugs with different forms of action is available to improve glycemic control. One drug widely used in the treatment of type 2 diabetes is metformin, a potent antihyperglycemic drug that suppresses hepatic gluconeogenesis [18,19], increases muscular glucose uptake [20,21] and ameliorates insulin sensitivity [22]. Furthermore, it has been shown that metformin improves the vascular function and reduces micro- and macrovascular complications [17,23].

This review provides an overview of the antihyperglycemic actions and clinical effects of metformin and presents some of the known molecular mechanisms underlying the beneficial metabolic effects of this antidiabetic drug. Furthermore, we will present evidence for the protective effects of metformin particularly in the brain and vasculature. The last part of this review culminates with a brief summary of the adverse effects inherent to metformin therapy.

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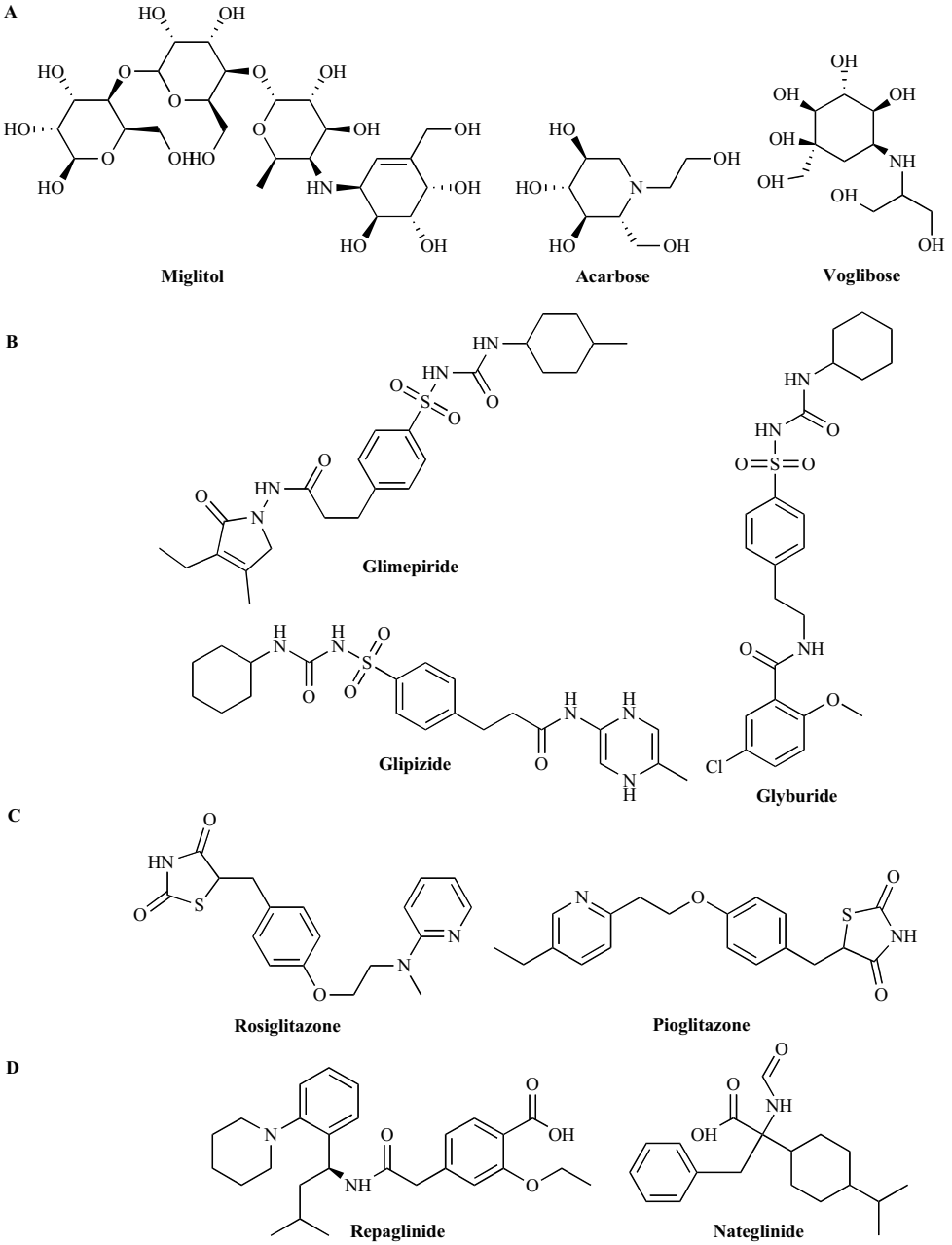

Fig. (1). Chemical structures of α-Glucosidase inhibitors (acarbose, miglitol and voglibose) (**A**), sulfonylureas (glyburide, glipizide, and glimepiride) (**B**), thiazolidinediones (rosiglitazone and pioglitazone) (**C**) non-sulfonylurea secretagogues (repaglinide and nateglinide) (**D**).

ORAL ANTIHYPERGLYCEMIC AGENTS IN THE TREATMENT OF TYPE 2 DIABETES

Evidence from the literature shows that optimal glycemic control is implicated in the reduction of type 2 diabetes-associated complications [24]. Oral antihyperglycemic agents, used alone or in combination with other oral antihyperglycemic agents or insulin, are often a necessary pharmacological tool to improve glycemic control. Currently, various classes of oral antihyperglycemic agents are available to treat type 2 diabetes: α-glucosidase inhibitors, insulin secretagogues, insulin sensitizers and biguanides [25] (Fig. **1**). α-Glucosidase inhibitors are competitive, reversible inhibitors of pancreatic α-amylase and membrane-bound intestinal α-glucosidase hydrolase enzymes. These oral antihyperglycemic agents have as main action the delay of intestinal carbohydrate absorption. Therefore, this retards glucose entry into the systemic circulation and lowers postprandial glucose levels [25,26]. The class of insulin secretagogues is composed by 2 subclasses: sulfonylureas (e.g. glyburide, glipizide, and glimepiride) and non-sulfonylureas (e.g. repaglinide and nateglinide). Insulin secretagogues are responsible by the augment of circulating insulin levels in patients with a moderate degree of ß-cell dysfunction, since they mimic glucose to close adenosine triphosphate-sensitive potassium

(K_{ATP}) channels and, consequently, stimulate insulin secretion [25,27]. Concerning to the mechanism of action of insulin sensitizers (thiazolidinediones) (e.g. rosiglitazone and pioglitazone), these drugs bind to peroxisome proliferator-activated receptor gamma (PPARγ) in adipose tissue, which regulate the transcription of genes involved in carbohydrate and lipid metabolism, acting as agonists. So, the major effect of the thiazolidinediones is the improvement of insulin sensitivity [28,29]. Finally, biguanides lead to the suppression of glucose production and the enhancement in glucose uptake by peripheral tissues. Metformin (1,1-dimethylbiguanide) (Fig. **2**), is a biguanide derivate widely used in the treatment of type 2 diabetes. This antidiabetic drug normalizes glucose levels without stimulation of insulin secretion, therefore it is considered an insulin sensitizer [25,30]. Metformin and its mechanisms of action in type 2 diabetes and associated complications are the focus of this review.

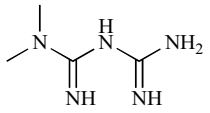

Fig. (2). Chemical structure of metformin (1,1-dimethylbiguanide).

CLINICAL EFFECTS OF METFORMIN

Type 2 diabetes is intimately associated with the development and progression of vascular complications [31]. Glycemic control along with hypertension and dyslipidemia management reduces micro- and macrovascular diseases, including cardiovascular events [24]. Additionally, it has been shown that glycemic control is a crucial factor in the reduction of microangiopathy, cardiovascular morbidity and mortality [16]. Metformin is one of the most effective antihyperglycemic agents, possessing the capability to lower glycosylated hemoglobin A_{1c} (HbA_{1c}) levels [32,33]. Several studies and randomized trials indicate that metformin therapy reduces cardiovascular morbidity and mortality in diabetic patients [34]. Furthermore, it has been reported that metformin treatment counteracts insulin resistance, reduces hyperinsulinemia, reduces body mass index and improves lipid profile, especially by reducing triglycerides and low density lipoproteins (LDL)-cholesterol levels and increasing high density lipoproteins (HDL)-cholesterol levels [35]. Moreover, the United Kingdom Prospective Diabetes Study (UKPDS) 34 showed that intensive treatment with metformin decreases macrovascular events and mortality in overweight diabetic patients, when compared with intensive treatment with exogenous insulin or sulphonylurea derivatives [17]. A recent clinical research study also showed that metformin treatment in people at risk for diabetes improves weight, lipid profiles, and insulin resistance, and reduces new-onset diabetes by 40% compared with placebo or no treatment [36]. Desilets and collaborators [37] also reported that the weight loss effects of metformin in overweight or obese adults and adolescents without diabetes appear promising. The same authors indicate that metformin may also have a positive effect on metabolic parameters such as waist circumference, fasting insulin and glucose levels and triglycerides [37]. Recently, Tan and collaborators [38] reported that metformin decreases serum vaspin (visceral adipose tissue-derived serine protease inhibitor) levels in overweight polycystic ovary syndrome women.

It has also been shown that in women with gestational diabetes mellitus, metformin (alone or with supplemental insulin) is not associated with increased perinatal complications as compared with insulin [39].

Altogether the above mentioned studies show that metformin treatment have several beneficial effects in prediabetic, diabetic and/or overweight patients.

THE ANTIHYPERGLYCEMIC ACTION OF METFORMIN

The antihyperglycemic effect of metformin relays in its ability to suppress gluconeogenesis and enhance glucose uptake and insulin sensitivity in peripheral tissues [40]. Therefore, this antidiabetic drug is capable to ameliorate insulin resistance and to reduce plasma glucose levels, which are crucial factors in the development of type 2 diabetes and associated complications.

Indeed, several studies demonstrated that metformin reduces glucose production mainly due to an inhibitory effect on gluconeogenesis [41-45]. Radziuk and collaborators [41] reported a decreased gluconeogenesis in perfused livers, essentially through inhibition of lactate uptake, by metformin. Furthermore, *in vitro* studies using isolated rat hepatocytes showed that metformin lowers intracellular levels of ATP, an inhibitor of pyruvate kinase [42]. Moreover, this antidiabetic drug also inhibits pyruvate carboxylase-phosphoenol-pyruvate carboxykinase (PEPCK) activity and activates the pyruvate to alanine conversion [46]. Despite the metformin's mechanisms of action in hepatocytes remain uncertain, the primary site of action of this drug appears to be mitochondria, since metformin inhibits mitochondrial respiratory chain particularly at the complex I level, impairing mitochondrial function and, consequently, cell function [47-49]. The inhibition of cellular respiration decreases gluconeogenesis [50] and enhances the expression of glucose transporters, stimulating glucose uptake. Further, the insulin receptor and the glucose transporters seem to be potential sites of action of metformin. A study performed in human hepatocytes showed that metformin quickly increases insulin receptor activation and signaling, essentially through insulin-receptor substrate-2 (IRS-2), and improves glucose transport through increased GLUT-1 translocation [51]. Besides the effect of metformin in gluconeogenesis, some studies also indicate that metformin reduces glycogenolysis [41]. Evidence from the literature also demonstrates that metformin enhances insulin-mediated glucose uptake [40]. It was observed that metformin normalizes insulin-mediated glucose disposal and muscle glycogen synthesis in diabetic rats [52]. Furthermore, *in vitro* studies also demonstrated the ability of metformin to increase glucose uptake in skeletal muscle [20,21,52,53]. This finding has been associated with increased insulin receptor tyrosine kinase activity [52], enhanced glycogen synthesis [54], and increased GLUT-4 transporter number and activity [55]. Although the mechanism that leads to GLUT-4 translocation is unclear, studies in different cell types [56-58] demonstrated that this antihyperglycemic drug increases insulin receptor binding tyrosine kinase activity [55], and insulin receptor

internalization [59]. It has also observed that metformin improves abnormal insulin receptor tyrosine kinase activity in muscle from streptozotocin-induced diabetic rodents [52].

Elevated plasma free fatty acids (FFA) play an important role in the establishment of insulin resistance. Chronic elevation in plasma FFA levels is commonly associated with impaired insulin-mediated glucose uptake in skeletal muscle and often coexists with obesity and type 2 diabetes [60,61]. Furthermore, increased plasma FFA concentration exerts a lipotoxic effect on the β-cell [62]. It has been attributed a reduction in FFA oxidation to metformin treatment [63]. In type 2 diabetic patients, metformin leads to the suppression of FFA and lipid oxidation [19]. However, this decrease seems to be contradictory to metformin action. Metformin induces the activation of AMP-activated protein kinase (AMPK), therefore it would be expected a stimulation of fatty acid oxidation instead of suppression. It has also been reported that chronic metformin treatment results in the lowering of lipids in human skeletal muscle [64,65]. Moreover, metformin treatment is frequently associated with a reduction in circulating triglycerides as a consequence of decreased synthesis and increased clearance of very low-density lipoproteins (VLDL) [66,67]. Further, a study in human pancreatic islets showed that metformin exerts a protective effect against lipotoxicity [68]. So, reduction in the concentration of plasma FFA can contribute to the improvement in insulin action and may also help to correct impaired insulin secretion by β-cells [69].

It has also been reported that metformin has a significant effect on the digestive tract by inducing a decrease in intestinal absorption of glucose [70,71], which could reduce postprandial blood glucose levels [60]. It has been hypothesized that increased glucose consumption in the small intestine of metformin-treated patients may prevent further glucose transport to the hepatic circulation [70].

In summary, metformin ameliorates hyperglycemia and insulin resistance through the suppression of gluconeogenesis, glycogenolysis and intestinal glucose absorption, reduction of FFA, and by the improvement in glucose uptake [40] (Fig. **3**).

POTENTIAL MOLECULAR MECHANISMS OF METFORMIN ACTION

Although the molecular mechanism underlying metformin action remains unclear, it has been suggested that this drug activates AMPK, a major regulator of cell and body energy homeostasis, by increasing its phosphorylation state but without any changes in AMP/ ATP ratio [72-74] (Fig. **4**). Recent studies demonstrated that serine-threonine kinase 11 (STK11/LKB1), which phosphorylates AMPK, is also a target of metformin [75,76]. Activation of AMPK leads to the inhibition of ATP consuming pathways and enhance ATP production pathways [77]. Indeed, the increase in AMPK activity is associated with the translocation of GLUT-4 to the membrane, the stimulation of glucose uptake in muscle and liver, glycolysis, fatty acid oxidation, and suppression of gluconeogenesis, glycogen, fatty acid and cholesterol synthesis [78] (Fig. **5**). It was showed that activation of AMPK by metformin is crucial for the decrease in glucose production and the increase in fatty acid oxidation in hepatocytes and for the increase in glucose uptake in muscle [73].

The main biological effects of AMPK are the phosphorylation and inactivation of acetyl-CoA carboxylase (ACC), which plays a pivotal role in hepatic lipid metabolism [79] (Fig. **4**). It was observed in cultured human hepatoma HepG2 cells that the stimulation of ACC phosphorylation by metformin induces the reduction in triglyceride levels, which can be supported with increased fatty acid oxidation and/or decreased fatty acid synthesis [80].

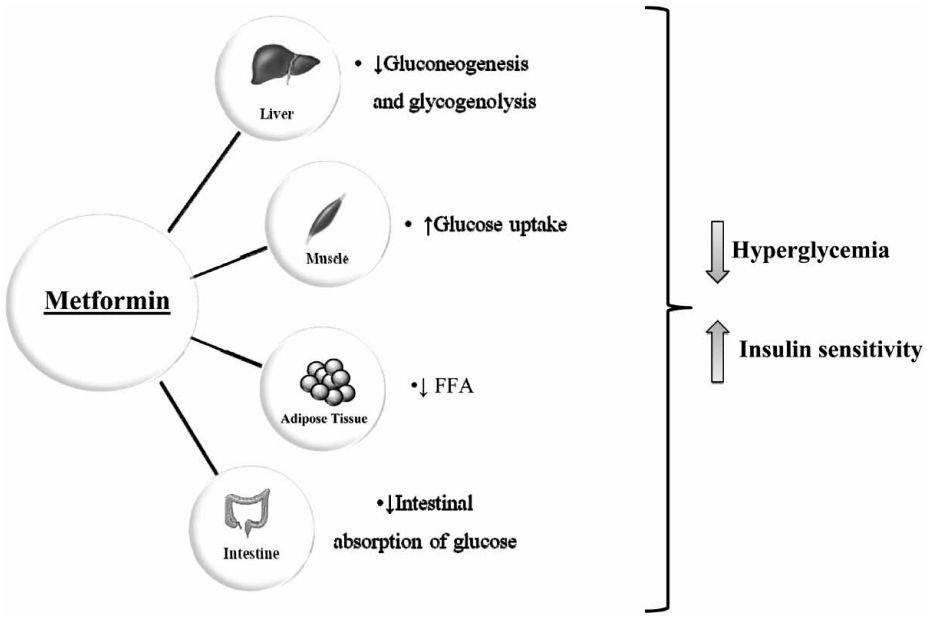

Fig. (3). Antihyperglycemic action of metformin. Metformin ameliorates hyperglycemia and enhances insulin sensitivity through the suppression of gluconeogenesis and glycogenolysis in liver, stimulation of glucose uptake in muscle, suppression of free fatty acid (FFA) and reduction of intestinal absorption of glucose.

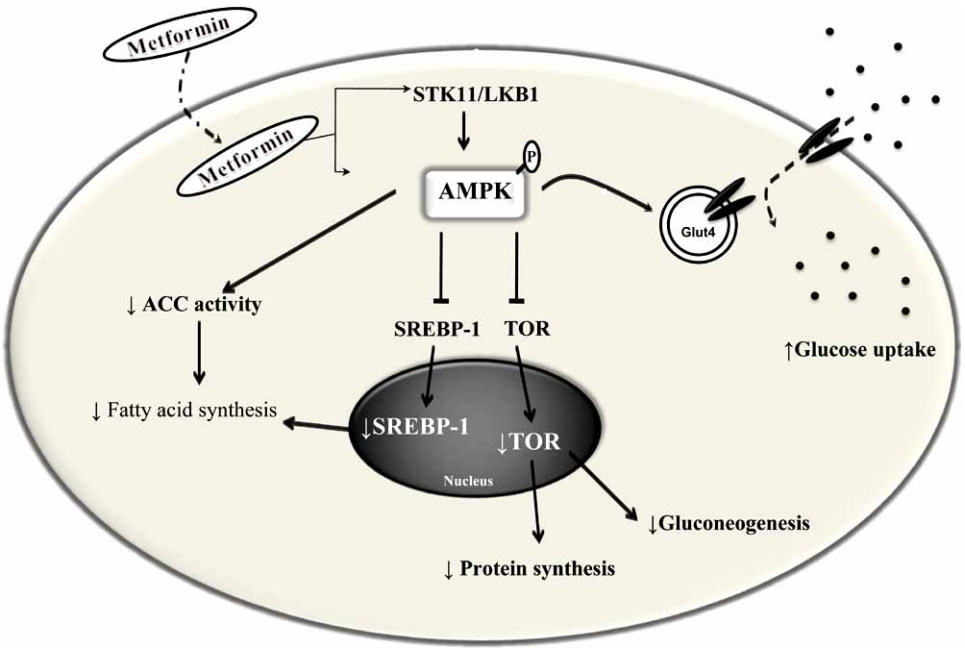

Fig. (4). Potential molecular mechanisms of metformin action. Metformin activates AMP-activated protein kinase (AMPK) by increasing its phosphorylation state and serine-threonine kinase 11 (STK11/LKB1) seems to be implicated in this process. Increased AMPK activity is associated with the translocation of glucose transporter (GLUT4) to the membrane and with the stimulation of glucose uptake. Further, AMPK inactivates acetyl-CoA carboxylase (ACC), decreasing fatty acid synthesis. Activation of AMPK by metformin also reduces the expression of sterol response element binding protein-1 (SREBP-1), a transcription factor that induces the expression of lipogenic genes, favoring the inhibition of fatty acid synthesis. Additionally, the activation of AMPK also promotes the inhibition of target of rapamycin (TOR) pathway, and, consequently, suppresses gluconeogenesis and protein synthesis.

Furthermore, AMPK is also implicated in gene regulation. Activation of AMPK by metformin reduces the expression of sterol response element binding protein-1 (SREBP-1), a transcription factor which induces the expression of lipogenic genes, including fatty acid synthase (FAS) and Spot-14 (S14) [73] (Fig. **4**). It has been postulated that SREBP-1 is a crucial mediator of insulin resistance in type 2 diabetes and associated metabolic disorders [81, 82]. Metformin's effects to modulate circulating lipids and to reduce hepatic lipid synthesis and fatty liver may be promoted by the reduced expression of SREP-1 induced by this antidiabetic drug [73]. Metformin through the activation of AMPK, inhibits the target of rapamycin (TOR) pathway [83,84], which could explain the suppression of gluconeogenesis and the increase in life extension observed in type 2 diabetic patients treated with metformin (Fig. **4**). Further, AMPK inhibits protein synthesis in many cells through the inhibition of TOR pathway [85].

Moreover, Shu and co-workers demonstrated that the organic cation transporter 1 (OCT1) is also involved in the antidiabetic action of metformin. It was observed a decrease in the effects of metformin both in AMPK phosphorylation and gluconeogenesis in mouse hepatocytes that harbor the *Oct1* deletion [86]. Further, a recent study suggested that plasma monoamine transporter (PMAT), expressed in human intestine, plays a role in the intestinal uptake of metformin [87].

In summary, the activation of AMPK may explain the metabolic benefits and lifespan extension properties of metformin.

VASCULOPROTECTIVE EFFECTS OF METFORMIN

The pathogenesis of type 2 diabetes markedly increases the incidence of vascular complications [88,89]. It has been postulated that this disorder is associated with an increased prevalence of vascular risk factors, including hyperglycemia, hypertension and dyslipidemia [90-92]. Indeed, insulin resistance and hyperinsulinemia, present in type 2 diabetes, contribute for impaired fibrinolysis, inflammation and oxidative stress [93,94]. Furthermore, hyperglycemia is responsible for the induction of cellular damage in endothelial cells. The structural and functional integrity of the endothelium plays a critical role in vascular homeostasis. Therefore, endothelial dysfunction, a hallmark of type 2 diabetes, is intimately involved in the onset of diabetic vascular complications [95,96]. It has been described that metformin improves vascular functions and dramatically reduces the incidence of vascular complications [97,98].

The improvement of glycemic control in type 2 diabetes could be beneficial to prevent diabetes-related vascular complications. Long-term control of blood glucose levels in type 2 diabetic patients may decrease the incidence and retard the development of diabetic retinopathy, nephropathy and neuropathy [99]. Furthermore, it has been reported that hyperglycemia, whether measured by HbA_{1C}, fasting or post-challenge glucose levels, is a cardiovascular risk factor [100-102]. In fact, several studies showed that metformin improves glycemic control, leading to a reduction of HbA_{1C} [34,36,103,104].

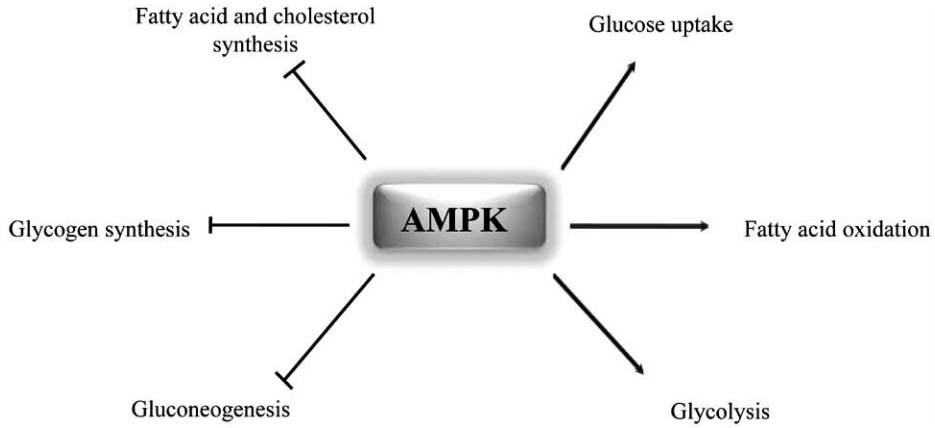

Fig. (5). Regulation of energy metabolism by AMP-activated protein kinase (AMPK). Activation of AMPK inhibits fatty acid and cholesterol synthesis, gluconeogenesis and glycogen synthesis. On the other hand, AMPK stimulates glucose uptake, glycolysis and fatty acid oxidation.

Diabetic dyslipidaemia is characterized by elevated triglycerides, HDL-cholesterol and accumulation of small dense LDL particles, which are particularly susceptible to oxidation. Oxidation of LDL leads to its uptake by monocytes, resulting in foam cell formation [105]. The generation of oxidized LDL is increased in diabetes as a consequence of both increased reactive oxygen species (ROS) generation and impaired antioxidant system [106]. In type 2 diabetic patients, metformin treatment induces decreased levels of plasma LDL cholesterol [36,107,108], increased in HDL cholesterol levels [36,60,109,110] and decreased levels of total cholesterol [36,108,109]. Moreover, metformin dramatically decreases plasma VLDL levels [109,111]. Finally, it has been shown that this oral antihyperglycemic drug decreases plasma FFA [109,112], and lowers lipid oxidation [22,113].

Type 2 diabetic patients exhibit increased platelet aggregation and plasma levels of clotting factors [111]. Furthermore, elevated levels of plasminogen activator inhibitor 1 (PAI-1) were described in different insulin resistant conditions, including type 2 diabetes. These elevated levels of PAI-1 result in impaired fibrinolysis and increased risk of thrombosis [114-123]. It has been reported that metformin increases fibrinolysis in patients with type 2 diabetes [124] mainly due to a decrease in PAI-1 levels in plasma [33,103,125]. Furthermore, this drug affects the coagulation system inducing a reduction in fibrinogen [126] and von Willebrand factor (vWF) levels [127]. Additionally, metformin was also associated with a reduction in coagulation factor VII levels [128]. A decrease in platelet aggregation was also observed in diabetic patients under metformin treatment [129]. Furthermore, *in vitro* and *in vivo* studies demonstrated that this antihyperglycemic drug improves vascular reactivity to the endothelium-dependent vasodilator acetylcholine in insulin resistant rats [130,131]. It has also been described that metformin improves blood flow [132]. Metformin increases haemodynamic responses to L-arginine, the precursor of vasodilatory nitric oxide (NO) [129], an effect that coud be explained by the observation that metformin lowers levels of asymmetric dimethylarginine (ADMA), in patients with type 2 diabetes [133]. ADMA is a mediator of endothelial dysfunction and a marker of vascular disease that is intimately involved in the pathogenesis of hypertension and atherosclerosis. Recently, it was demonstrated that clinically related concentrations of metformin activate endothelial nitric oxide synthase (eNOS) and NO bioactivity in an AMPK-dependent manner [134]. Finally, Mamputo and collaborators [135] reported that metformin is able to inhibit monocyte adhesion to human endothelial cells and foam cell formation.

The formation of AGEs has been considered an important mediator in diabetes-related complications. Under hyperglycemic conditions AGEs accumulate in the vessel wall, where they may alter cell structure and function. The recognition and binding of AGEs to the receptor for AGEs (RAGE) contribute to the micro- and macrovascular complications of diabetes [136]. AGEs and activation of RAGE on endothelial cells increases oxidative stress and inflammatory processes. It has been shown that metformin reduces both oxidative stress and C-reactive protein (PCR) levels [137-139]. Metformin also reduces the levels of soluble intercellular cell-adhesion molecules (ICAM-1) and soluble vascular cell-adhesion molecules (VCAM-1) in subjects with impaired glucose tolerance [140]. Moreover, it was reported that metformin decreases the levels of soluble VCAM-1 and soluble E-selectin in diabetic patients, independent of its effects on glucose levels [141]. The increased levels of soluble forms of these molecules have been associated with increased risk of coronary events [142-144]. Furthermore, *in vitro* studies demonstrate that metformin prevents formation of AGEs and AGE cross-linking [145] in a way independent of its antihyperglycemic effects. More recently, Ouslimani and co-workers [146] reported that metformin also inhibits the cell expression of both RAGE and lectin-like oxidized receptor 1 (LOX-1), two endothelial receptors involved in the arterial endothelial dysfunction. *In vitro* studies using bovine aortic endothelial cells (BAEC) also shown that metformin decreases the intracellular production of ROS [147,148] through the inhibition of PKC [148]. Further, an *in vivo* study performed in Goto-Kakizaki (GK) rats, a model of type 2 diabetes, demonstrated that metformin delays the manifestation of diabetes and vascular dysfunction by the reduction of mitochondrial oxidative stress [139]. Finally, it was reported that metformin exerts a cardioprotective effect through the Akt-mediated inhibition of mitochondrial permeability transition pore (mPTP) opening [149]. Accord-

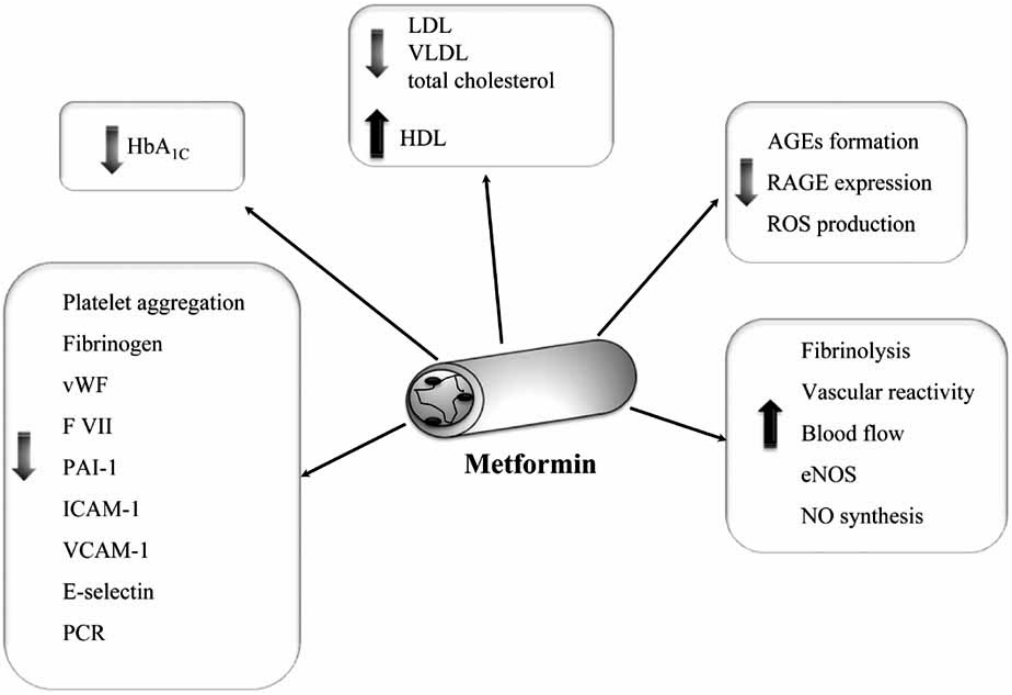

Fig. (6).Vasculoprotective effects of metformin. Metformin is able to reduce glycosylated hemoglobin A_{1C} (HbA_{1C}) , low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total cholesterol, plasminogen activator inhibitor 1 (PAI-1), von Willebrand factor (vWF), factor VII and C-reactive protein (PCR), soluble endothelial adhesion molecules (vascular cell-adhesion (VCAM), intercellular cell-adhesion molecules (ICAM) and E-selectin) and fibrogen levels, platelet aggregation, blood flow, formation of advanced glycation end products (AGEs), expression of receptor for advanced glycation end products (RAGE) and reactive oxygen species (ROS). In contrast, metformin enhances fibrinolysis, vascular reactivity and high-density lipoprotein (HDL) cholesterol levels. Metformin activates endothelial nitric oxide synthase (eNOS) and stimulates nitric oxide (NO) synthesis. Therefore, metformin has a protective action at the vascular level.

ingly, it was also observed that metformin has the capacity to regulate mPTP opening in endothelial cells exposed to high glucose levels and, consequently, prevents high-glucose-induced endothelial cell death [150].These evidences support the idea that metformin, beyond its antihyperglycemic action, possesses antioxidant properties.

In conclusion, metformin ameliorates vascular function and reduces the incidence of diabetes-associated vascular complications by the improvement of glycemic control, insulin resistance, lipid profile, fibrinolitic activity, oxidative stress and endothelial function. Altogether, these findings suggest that metformin has a potent protective action on vascular cells (Fig. **6**).

COULD METFORMIN EXERT A PROTECTIVE EFFECT IN THE BRAIN?

Diabetes mellitus is associated with slowly progressive changes in the brain [151]. An impaired cognitive functioning and an increased risk of dementia and neurodegeneration have been reported in patients with type 2 diabetes [152-155]. The mechanism through which type 2 diabetes affects the brain remains uncertain however hyperglycemia, insulin resistance, oxidative stress, AGEs formation, inflammatory cytokines and vascular complications may be involved [152,156].

Recent data from our laboratory showed that metformin, beyond its antihyperglycemic properties, is capable to protect the brain of diabetic GK rats against diabetes-associated oxidative stress [157]. Indeed, we observed that metformin treatment decreases lipid peroxidation, increases reduced glutathione (GSH) levels and normalizes the activities of antioxidant enzymes suggesting that this oral antidiabetic drug could be a neuroprotective agent [157]. Accordingly, El-Mir and collaborators [158] attributed a neuroprotective role to metformin and purposed that this drug could function as a therapeutic tool for diabetes-associated neurodegenerative disorders. In this work it was observed that metformin inhibits the mPTP opening in primary cortical neurons. It has also been shown that metformin protects rats against cerebral ischemia [159].

Since more than 20 syndromes among the significant and increasing number of degenerative diseases of neuronal tissues are known to be associated with diabetes mellitus, increased insulin resistance and obesity, disturbed insulin sensitivity, and excessive or impaired insulin secretion [160], the potential neuroprotective effects of metformin could be of extreme importance. Indeed, a recent study demonstrated that metformin therapy significantly prolonged survival in a transgenic mouse model of Huntington's disease (HD) [161].

As previously discussed, metformin induces beneficial changes in glycemic control, insulin resistance, lipid profile and vascular complications that altogether can contribute for a positive impact of this drug at the brain level.

ADVERSE EFFECTS OF METFORMIN THERAPY

It has been reported that biguanidines (metformin and phenformin) reduce pyruvate dehydrogenase activity and

mitochondrial transport of reducing agents enhancing anaerobic metabolism [162]. This shift to anaerobic metabolism, in the presence of reduced insulin, increases production of precursors for the Krebs cycle [163]. The inhibition of pyruvate dehydrogenase results in a decreased ability to channel those precursors into aerobic metabolism, which, in turn, results in increased metabolism of pyruvate to lactate and increases the net lactic acid production. Additionally, increased glucose utilization in the small intestine caused by biguanides could theoretically increase portal vein lactate levels. Phenformin and metformin have a similar mechanism of action but phenformin has a much greater propensity to cause lactic acidosis [163]. Because of the high incidence of lactic acidosis associated with phenformin, this antidiabetic agent was removed from clinical use. Compared with metformin, patients taking phenformin have a 10 to 20 times greater risk of developing lactic acidosis [22]. Due to its higher lipophilicity, phenformin has a greater affinity for binding to mitochondrial membranes, which could account for its greater ability to inhibit aerobic metabolism than metformin [162]. Additionally, after phenformin was withdrawn from the market, it was found that certain patients have an inherited defect in hydroxylation of the drug [164] that could have resulted in phenformin accumulation. Metformin, in contrast, is not metabolized. The overall average estimated incidence of metformin-induced lactic acidosis is rare: 0.03 cases per 1000 patient-years [22,162,165]. Indeed, a recent review show that metformin therapy is not associated with increased levels of lactate and risk of lactic acidosis [166]. However, metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including kidney disorders (creatinine levels over 150 μmol/l [167], lung disease and liver disease. Heart failure has long been considered a contraindication for metformin use, although a 2007 systematic review showed metformin to be the only antidiabetic drug not associated with harm in people with heart failure [168].

The most common adverse effects of metformin are restricted to digestive tract symptoms such as diarrhea, flatulence, and abdominal discomfort [169-171]. In a clinical trial of 286 subjects, 53.2% of the 141 who were given immediate-release metformin (as opposed to placebo) reported diarrhea, versus 11.7% for placebo and 25.5% reported nausea/vomiting, versus 8.3% for those on placebo [172]. This antidiabetic drug has also been implicated with increased homocysteine levels [173] and vitamin B_{12} deficiency [174-176]. In fact, it has been demonstrated that 10-30% of diabetic patients that received long-term metformin therapy develop vitamin B_{12} malabsorption, indicated by reduced concentrations of total vitamin B_{12} and its bioavailable form, holotranscobalamin. This antidiabetic agent interferes with mucosal-cell intracellular calcium handling, leading to a disruption in calcium-dependent absorption of vitamin B_{12} in the ileum [36,177]. Furthermore, it was reported 2 clinical cases of possible metformin-induced hepatotoxicity; both patients developed cholestatic jaundice after the initiation of metformin treatment suggesting that, although uncommon, metformin can induce liver damage/hepatotoxicity [178, 179]. A recent study showed also that relatively high levels of metformin induce liver mitochondria injury [180] supporting the idea that the chronic use of this antidiabetic agent may predispose to hepatotoxic injury [178,179].

Despite the above mentioned side effects, the benefits of metformin treatment are clearly superior and nowadays this drug is widely used in the management of type 2 diabetes.

CONCLUSION

Vascular complications are the main cause of premature mortality and morbidity in type 2 diabetes. Furthermore, type 2 diabetes is also related with impaired cognitive functioning and increased risk of dementia and neurodegeneration. However, the incidence of diabetic complications can be reduced by tight glycemic control. Metformin is widely used in the management of type 2 diabetes. The primary actions of this antihyperglycemic drug are suppression of gluconeogenesis and improvement of glucose uptake and insulin sensitivity. It has been reported that the activation of AMPK mediates beneficial metabolic effects of metformin. Indeed, the activation of AMPK by metformin induces translocation of glucose transporter (GLUT-4) to the membrane, stimulates glucose uptake, and suppresses gluconeogenesis and fatty acid and cholesterol synthesis. Additionally, it has been postulated that metformin improves vascular functions and dramatically reduces micro- and macrovascular complications associated to diabetes. Indeed, metformin is able to reduce the levels of HbA_{1C}, LDL particles, total cholesterol, PAI-1, vWF, factor VII, PCR, soluble endothelial adhesion molecules and inhibits platelet aggregation and formation of AGEs. On the other hand, metformin enhances fibrinolysis, vascular reactivity, blood flow and HDL cholesterol levels. Furthermore, recent studies reported that metformin exhibits antioxidant properties that contribute to ameliorate oxidative stress and, therefore, to attenuate the deleterious effects of type 2 diabetes. In fact, this antidiabetic drug has the ability to reduce ROS production in endothelial cells and to protect the brain against diabetes-associated oxidative stress. In summary, it is notorious that metformin is a potent antihyperglycemic drug able to counteract some risk factors involved in the onset and progression of diabetes-associated complications.

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