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New Insight into the Mechanisms of the Anti-hyperglycemic Action of Metformin

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Author's contribution

The sole author performed the literature search, designed and wrote the manuscript.

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ABSTRACT

Although metformin is currently one of the most frequently prescribed drugs for the treatment of type 2 diabetes, the precise mechanism of its molecular action is not fully understood. Metformin induces mild and transient inhibition of mitochondrial respiratory-chain complex I activity, resulting in the activation of adenosine monophosphate-activated protein kinase (AMPK) and suppression of hepatic gluconeogenesis. However, recent studies provide evidence that several AMPK-independent pathways may be involved in the action of metformin.

The aim of this review is to summarize novel findings on the mechanisms of the anti-hyperglycemic action of metformin, with a special attention paid to AMPK-independent pathways. The results of recent studies with gut-restricted delayed-release metformin formulation demonstrating dissociation of its glucose-lowering effect from plasma exposure are also discussed. The role of the gastrointestinal tract in the action of metformin is summarized focusing on the enhanced secretion of glucagon-like peptide-1 and modulation of gut microbiota.

Recent scientific evidence extends our understanding of the complex mechanisms of metformin action, points towards potential new molecular targets for the treatment of diabetes and may promote the development of new antidiabetic therapies.

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Keywords: Metformin; type 2 diabetes; adenosine monophosphate-activated protein kinase; glucagon signaling; glucagon-like peptide-1; gut microbiota.

LIST OF ABBREVIATIONS

AMP – adenosine monophosphate; AMPK - 5'-AMP-activated protein kinase; ATP - adenosine triphosphate; cAMP- cyclic AMP; DPP-4 - dipeptidyl peptidase-4; GIP - glucose-dependent insulinotropic peptide; GLP-1 - glucagon-like peptide-1; IGF-1 - insulin-like growth factor 1; Met DR - delayed-release metformin; Met XR - extended-release metformin; mGPD - mitochondrial glycerol-phosphate dehydrogenase; mTORC1 - mammalian target of rapamycin complex 1; NADH - nicotinamide adenine dinucleotide; OCT1 - organic cation transporter; PKA - protein kinase A; PYY - peptide YY; SREBP-1 - sterol regulatory element-binding protein-1

1. INTRODUCTION

Metformin is currently the drug of choice in patients with type 2 diabetes mellitus, as indicated in the guidelines published by the European Association for the Study of Diabetes and American Diabetes Association [1]. Although metformin has been used as a treatment for type 2 diabetes since 1950s, the molecular mechanism of its action has not been fully elucidated yet. The glucose-lowering effect of metformin has been mainly attributed to its ability to suppress hepatic gluconeogenesis [2], although some studies have reported increase in peripheral glucose uptake [3]. Since a key study by Zhou et al. [4] in 2001, it is has been generally accepted that metformin activates adenosine monophosphate-activated protein kinase (AMPK), a master kinase regulating cellular energy homeostasis, resulting in suppression of hepatic gluconeogenesis, increase of fatty acid oxidation and suppression of a key lipogenic transcription factor, sterol regulatory element-binding protein-1 (SREBP-1), expression. The study by Shaw et al. [5] has shown that LKB1 tumor suppressor is the major upstream activating kinase for AMPK that mediates glucose homeostasis in liver and therapeutic effects of metformin. It has been reported that AMPK phosphorylates cAMP-response element binding protein (CREB)-regulated transcription coactivator 2 (TORC2), resulting in its inactivation which consequently downregulates transcription of gluconeogenic enzymes [5]. However, TORC2 is O-glycosylated at Ser^{171} in insulin resistance state, making phosphorylation impossible [6]. Alternatively, as proposed by Caton et al. [7], metformin inhibits TORC2-mediated gluconeogenesis through induction of hepatic SIRT1. SIRT1, an NAD^+-dependent protein deacetylase, inhibits gluconeogenesis through disruption of TORC2 signalling. Several SIRT1 activators have been demonstrated to improve glucose tolerance and enhance glucose-stimulated insulin secretion in animal models [8], however, these beneficial effects have not yet been confirmed in clinical trials in patients with type 2 diabetes [9].

Recently, the only genome-wide pharmacogenetics study with metformin has identified a variant in *ATM* gene to be associated with response to metformin [10]. Mutation in *ATM* causes ataxia-teleangiectasia, an inherited disease associated with diabetes. ATM protein plays a role in DNA repair and has been shown also to activate AMPK.

Further studies have demonstrated that AMPK activation by metformin is secondary to its effect on the mitochondrial respiratory-chain complex I. It has been shown that mitochondrial respiratory-chain complex I is the primary target of metformin, as was originally proposed by Owen et al. [11], and the specific AMPK-independent inhibition of the mitochondrial respiratory-chain complex I by metformin leads to a time- and dose-dependent decrease in adenosine triphosphate (ATP) levels, which results in a concomitant increase in adenosine monophosphate (AMP) intracellular levels (and the AMP: ATP ratio), triggering the activation of AMPK [12].

The AMPK-dependent mechanism involved in the suppression of glucose production and gluconeogenic gene expression by metformin, has been recently confirmed in the study by Cao et al. [13]. However, studies in AMPK- and LKB-1-deficient hepatocytes have demonstrated that metformin can also decrease hepatic gluconeogenesis independently of the LKB-1/AMPK pathway. There is also growing evidence suggesting that the gastrointestinal tract may play an important role in the action of metformin.

2. AMPK-INDEPENDENT PATHWAYS INVOLVED IN THE ACTION OF METFORMIN

Using genetic mouse models, Foretz et al. [14] have demonstrated that metformin inhibits gluconeogenesis through LKB1- and AMPK-independent pathways, and that metformin may inhibit glucose production independent of direct inhibition of gluconeogenic gene expression, acting via a decrease in hepatic energy state. A recent study by Madiraju et al. [15] has shown that one of the primary molecular targets by which metformin inhibits hepatic gluconeogenesis is the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase (mGPD). According to their study, metformin non-competitively inhibits mGPD, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose, and decreased hepatic gluconeogenesis.

Miller et al. [16] have recently proposed a novel AMPK-independent mechanism related to glucagon signaling by which metformin acutely inhibits glucose production. Glucagon is released in response to fasting or starvation and acting via cAMP and cAMP-dependent protein kinase A (PKA), promotes hepatic glucose production by inhibiting glycolysis and activating gluconeogenesis. Relative glucagon hypersecretion in the fasted state and the lack of suppression of postprandial glucagon secretion contribute to the increased hepatic glucose output in patients with type 2 diabetes. Miller et al. [16] have demonstrated in mouse hepatocytes that metformin leads to the accumulation of AMP and related nucleotides, which inhibit adenylate cyclase, decrease levels of cyclic AMP and PKA activity, abrogate phosphorylation of critical protein targets of PKA, and block glucagon-dependent hepatic glucose output (Fig. 1). These data support a novel mechanism of metformin action involving antagonism of glucagon, and a potential role of adenylate cyclase as a new target for the treatment of type 2 diabetes.

Taking into account the growing appreciation of the pathophysiologic importance of diabetic hyperglucagonemia [17], antagonizing glucagon action represents an attractive therapeutic option in patients with diabetes. There is ongoing research on glucagon receptor antagonists as potential treatment for patients with type 2 diabetes [18], however, several safety issues have been raised, including the increase in recovery time from hypoglycemia, elevated liver transaminases and LDL cholesterol [19]. Moreover, uncontrolled alpha-cell growth and development of pancreatic neuroendocrine tumors in mice lacking functional glucagon receptor has been reported [20]. Thus, for now, blockage of glucagon signalling by inhibiting adenylate cyclase with metformin represents a safe option to reduce the inappropriate glucagon secretion in type 2 diabetes.

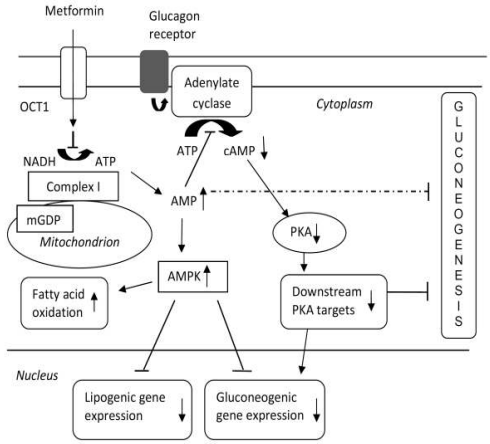

Fig. 1. Potential mechanisms underlying the action of metformin on hepatic gluconeogenesis

Metformin is transported into the hepatocyte via the organic cation transporter (OCT1). Metformin inhibits complex I of the respiratory chain and mitochondrial glycerol-phosphate dehydrogenase (mGPD), resulting in decreased ATP synthesis and an accumulation of AMP. AMP inhibits the activity of adenylate cyclase, leading to reduced generation of cAMP upon stimulation of the glucagon receptor, thus inhibiting PKA activation. As a result, the ability of PKA to promote gluconeogenesis is abrogated. Gluconeogenesis is suppressed as a result of reduced gluconeogenic gene expression and reduced activity of gluconeogenic enzymes.

Increased AMP intracellular levels activate AMPK, which suppresses lipogenesis and contributes to the reduced gluconeogenic gene expression. A decrease in ATP and a concomitant increase in AMP may also contribute to a direct inhibition of gluconeogenesis; OCT1 - organic cation transporter; mGPD - mitochondrial glycerol-phosphate dehydrogenase; NADH - nicotinamide adenine dinucleotide; ATP - adenosine triphosphate; AMP – adenosine monophosphate; cAMP- cyclic AMP; PKA - protein kinase A; AMPK - 5'-AMP-activated protein kinase

3. LOWER BOWEL-MEDIATED MECHANISM OF METFORMIN ACTION

It has been shown that intravenous metformin administration is less effective than oral administration, suggesting that gastrointestinal tract may predominantly account for the glucose-lowering effect of metformin [21,22]. After oral administration of currently available metformin formulations (immediate-release metformin and extended-release metformin), the bioavailability is approximately 50% of the total dose, and the majority of absorption occurs in the duodenum and jejunum [23]. Metformin is not metabolized and is excreted in the urine and bile in an unmodified form. Metformin is supplied to the liver directly from the gut via the portal vein, enters hepatocytes through the organic cation transporter-1 (OCT-1) and is accumulated in the liver at concentrations approximately 10 times higher than those in plasma [24]. However, metformin also accumulates in the gut mucosa at concentrations 300 times greater than in plasma [25]. Recently Buse et al. [26] have demonstrated that administration of 1000 mg of delayed-release metformin formulation (Met DR) that targets the ileum, where the absorption of metformin is low, resulted in a 50% greater median reduction in plasma glucose levels than administration of 1000 mg of extended-release metformin (Met XR) in patients with type 2 diabetes over 12 weeks. Met XR was administered once a day with the evening meal, and twice-daily doses of Met DR were administered after meals in order to reduce gastrointestinal adverse effects, such as abdominal pain, flatulence and diarrhea. The results this study [26] suggest a predominantly lower bowel-mediated mechanism of metformin action at therapeutic doses. The observation that metformin delivered to the lower bowel with Met DR acts with a glucose-lowering efficacy comparable to that of Met XR, but with significantly lower systemic exposure, is of important clinical significance, and if it is confirmed in further studies, a gut-restricted delayed-release metformin may be administered in patients with renal impairment without the risk of metformin-associated lactic acidosis.

4. METFORMIN AND GASTRO-INTESTINAL HORMONES

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are gastrointestinal hormones released in response to food intake. Both GLP-1 and GIP increase insulin secretion. GLP-1 also decreases glucagon secretion, and, as it has been shown recently, insulin stimulation and glucagon inhibition contribute equally to the glucose-lowering action of GLP-1 in patients with type 2 diabetes [27]. Moreover, GLP-1 improves insulin sensitivity, decreases hepatic gluconeogenesis and delays gastric emptying, potentially promoting central satiety [28]. Both GLP-1 and GIP have a half-life of less than 2 minutes, due to rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP-1 receptor agonists (exenatide, liraglutide, albiglutide, dulaglutide and lixisenatide) have been recently introduced in the treatment of diabetes [29].

It has been shown that metformin increases plasma levels of GLP-1, but not that of GIP [30,31]. It has been also demonstrated that metformin increases GLP-1 receptor expression on islet cells via a pathway dependent on peroxisome proliferator-activated receptor-alpha [31]. The mechanism underlying metformin-induced GLP-1 secretion has not been fully elucidated: Mulherin et al. [32] suggested the involvement of a non-vagal M3 muscarinic pathway, and Kim et. al. [33] reported that metformin enhanced GLP-1 production via cooperation between insulin and Wnt signaling.

Recently, Napolitano et al. [34] evaluated patients with type 2 diabetes on and off metformin monotherapy to characterize the gut-based mechanisms of metformin. They found that metformin withdrawal was associated with the reduction of active and total GLP-1. This effect was reversed when metformin was restarted. Effects on plasma levels of peptide YY (PYY), which co-localizes with GLP-1 in intestinal L cells were modest, and GIP secretion changes were negligible.

5. MODULATION OF GUT MICROBIOTA DURING METFORMIN TREATMENT

There is a growing evidence showing relationship between metabolic disorders, such as obesity and diabetes, and gut microbiome composition [35]. Transplantation of intestinal microbiota from obese mice to germ-free mice causes a significant increase in insulin resistance and body fat content [36]. It has been shown that the treatment with metformin in diet-induced obese mice modulates the gut microbiota by an increase in the *Akkermansia spp*. population [37]. *Akkermansia muciniphila* is a mucus-degrading

Gram-negative anaerobic bacteria that resides in the mucus layer. Oral administration of *Akkermansia muciniphila* to high-fat diet-fed mice had similar beneficial metabolic effects to that of metformin administration. It significantly enhanced glucose tolerance, increased the number of mucin-producing goblet cells and attenuated adipose tissue inflammation by reversing diminished regulatory T cell numbers and elevated interleukin 1β or IL-6 mRNA expression in the visceral adipose tissue [37]. *Akkermansia muciniphila* administration increased the intestinal levels of endocannabinoids that control inflammation, the gut barrier and gut peptide secretion [38]. Further animal studies demonstrated that in addition to the changes in the intestine microbiota associated with metformin treatment, several metabolic pathways (including those for sphingolipid and fatty acid metabolism) were significantly upregulated in the gut microbiota during metformin treatment [39].

Most bacterial species in the mouse and human gut belong to the phyla Bacteroidetes and Firmicutes. Recently, Napolitano et al. [34] have presented the first evidence that human intestinal microbiome in patients with type 2 diabetes changes when patients are on- or off-metformin. Metformin withdrawal was also associated with the elevation of serum bile acids, especially cholic acid and its conjugates. These effects were reversed when metformin was restarted. Microbiota abundance of the phylum Firmicutes was positively correlated with changes in cholic acid and conjugates, while Bacteroidetes abundance was negatively correlated. Firmicutes and Bacteroidetes representation were also correlated with levels of serum PYY. Previous animal studies have shown that obesity and high-fat diet are associated with a significant decrease in Bacteroidetes phylum and an increase in Firmicutes phylum [40]. Recently, Cabreiro et al. [41] have shown that metformin alters folate and methionine metabolism of the gut microbiota in the worm *Caenorhabditis elegans*, leading to a state of nutritional restriction, which increases lifespan.

6. METFORMIN AND GLUCOSE METABOLISM IN CANCER

There is substantial clinical evidence that patients with type 2 diabetes treated with metformin might have a lower cancer risk [42]. Preclinical data suggest that metformin inhibits proliferation and induces apoptosis in several types of cancer cells [43]. The anticancer molecular action of metformin has been associated with the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) that may be dependent [44] or independent on AMPK activation [45]. Inhibition of mTORC1 signaling leads to inhibition of protein synthesis and cancer cell proliferation. Metformin may also exert indirect inhibitory effect on cancer progression through lowering serum insulin level and consequently inhibiting insulin-like growth factor 1 (IGF-1) signaling pathways [46].

Metformin treatment increases the AMP: ATP ratio, switching cells from an anabolic to catabolic state. The results of recent *in vitro* studies with ^{18}F-fluoro-deoxy-glucose in different cancer cellular models indicate that activation of AMPK by metformin is associated with reduction in glucose uptake in cancer cells, thus limiting energy resources and affecting cancer cell proliferation [47,48]. A study in a mouse model of prostate cancer has shown that metformin decreases glucose oxidation and induces the dependency on reductive glutamine metabolism [49].

In the presence of oxygen, differentiated cells first metabolize glucose to pyruvate via glycolysis and then oxidize the pyruvate to carbon dioxide during the process of oxidative phosphorylation in the mitochondria. Regardless of the presence of oxygen, cancer cells tend to convert most of glucose to lactate during the process of aerobic glycolysis. This phenomenon is known as the Warburg effect [50]. Metformin impairs glycolysis and reduces the availability of cellular energy by decreasing the activity of enzyme hexokinase 2, which catalyses the glucose phosphorylation reaction – the first step in glucose metabolism [51].

7. CONCLUSION

It has been generally accepted that the anti-hyperglycemic action of metformin is primarily exerted in the liver, at least partly via the activation of AMPK and the subsequent inhibition of gluconeogenesis [43]. However, there is growing evidence demonstrating AMPK-independent mechanisms of metformin action [52,53]. Moreover, recent studies with gut-restricted delayed-release metformin formulation demonstrated dissociation of its glucose-lowering effect from plasma exposure, suggesting the predominant role of the gastrointestinal tract in the action of metformin [26]. The gastrointestinal

effects of metformin include increasing secretion of GLP-1, modulation of gut microbiota and alteration of the entero-hepatic recirculation of bile acids.

A considerable progress that has been made recently in our understanding of the complex mechanisms underlying the action of metformin, points towards potential new molecular targets for the treatment of diabetes and may result in the development of novel antidiabetic therapies. Furthermore, metformin has been lately of great interest in the field of oncology.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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