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Review Article

**METFORMIN: PROPOSED MODE OF ACTION IN TYPE 2
DIABETES**

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Abstract:

Anti hyperglycemic agents, the biguanide metformin is the brightest star, features as first-line pharmacologic treatment for type 2 diabetes in virtually all guidelines and recommendations, efficacy and tolerability of which are well tested, concluded with safety and affordable, most widely used anti diabetic drug, alone or in combination with other anti hyperglycemic agents. Unlike the sulfonylurea and insulin, metformin is not associated with weight gain or it is quite friendly to patients but its mechanism of action has been very difficult to pinpoint. This review will focus to explore all the level of proposed mechanism along with novel recruited in the field of medicine.

Key words: Antihyperglycemia, Biguanide, Metformin, Sulfonylurea, Insulin

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INTRODUCTION

Wealthy modernized industrial society has a commonly exhibited problem known as diabetes type 2 mellitus that comprise abnormalities in insulin action, particularly resistance [1] corresponds to elevated blood glucose level for which anti hyperglycemic agent, widely prescribed is metformin that reduces blood glucose level without increasing insulin secretion, and hence sometime named as an insulin sensitizer [2]. In fact, metformin showed beneficial effects in type 2 diabetes, including weight reduction, improved lipid profiles, and enhanced endothelial function [3] Thus, metformin is introduced for use in insulin-resistant state even before the development of hyperglycemia [4]. It is well documented that rate of hepatic glucose production was twice as high in diabetics than in controls and treatment with metformin reduced the rate of production by 24%, and fasting plasma glucose concentration by 30%, also the rate of gluconeogenesis is 3 times higher in diabetics which is lowered by metformin by 36%. In conclusion metformin lowers the rate of endogenous glucose production by multiple pathways including diminished gluconeogenesis [5,6,7].

Literature search strategies

Articles in various international and national bibliographic indices were extensively searched, with an emphasis on metformin, novel mode of action and management of type 2 diabetes. The various search sites included Entrez (including PubMed), Hinari free search and Medscape.com, WebMD.com, MedHelp.org, and google.com.

Selective Site of action of metformin

In animals and humans, pharmacokinetics of metformin is largely determined by its active transport through major organic cation transporters [8] across the gut epithelium and hence determine rates of absorption (plasma membrane monoamine transporter (PMAT) and organic cation transporter (OCT3), they transport metformin into hepatocytes (OCT1) and from hepatocytes into the bile (multidrug and toxic compound extrusion [MATE] 1) and, finally, into the renal tubular epithelial cells (OCT2) and into the renal tubule (MATE2) [9]. *Oct1* (also known as *Slc22a1*) knockout mice display reduced efficacy of Metformin [10] and this work has established an important role for OCT1 in metformin intake and augment the preferential role of the liver as the primary site of action for metformin, summarizing the effect whereby absorption through the upper small intestine concentrated in enterocytes and than to hepatocyte subsequently circulates freely, and is eliminated, without any alteration by the kidneys [11, 12]. The selective effects of metformin in hepatocyte is explained on the basis that deletion of the OCT1 gene in mouse dramatically reduces metformin uptake in hepatocytes and human individuals carrying an impaired effect of metformin in lowering blood glucose [13], predominant expression of the organic cation transporter 1 (OCT1) is present in the hepatocyte cell (Fig.1) that facilitate cellular uptake of Metformin [14].

Although the molecular target of metformin was elusive, Zhou *et al.* reported that AMP-activated protein kinase (AMPK) was solely associated with the pleiotropic actions of Metformin [15].

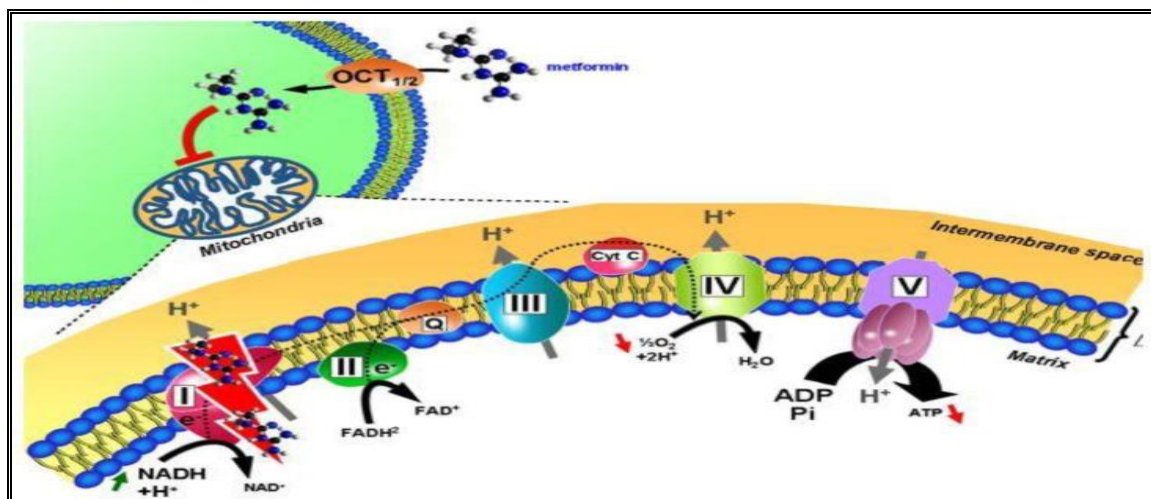


Fig1: Primary Site of Metformin Action.

Intracellular transport is mediated by different isoforms of the organic cation transporters (OCT) (e.g. OCT1 in liver or OCT2 in kidney). Once inside the cytosolic compartment, mitochondria then constitute the primary target of metformin to inhibit mitochondrial respiratory-chain specifically at the complex I level without affecting any other steps of the mitochondrial machinery. This unique property of the drug induces a decrease in NADH oxidation, proton pumping across the inner mitochondrial membrane and oxygen consumption rate, leading to lowering of the proton gradient ($\Delta\phi$) and ultimately to a reduction in proton-driven synthesis of ATP from ADP and inorganic phosphate (Pi).

Metformin and AMPK

It has been demonstrated that 5'AMP-activated protein kinase (AMPK) work as sensor of cellular energy status and is activated in situation of high energy phosphate depletion such as in muscle contraction during exercise and hypoxia, acting as a cellular mediator of metformin in hepatocytes has been considered to be a major signal in suppressing lipogenesis and inducing fatty acid oxidation [16], thereby reducing activity of acetyl-CoA carboxylase and lowering expression of a lipogenic transcription factor as well as inhibiting hepatic gluconeogenesis. Indeed plasma glucose-lowering action is not entirely dependent on insulin but also exercise causes an increase of glucose uptake in the skeletal muscle of diabetic and nondiabetic subjects through the translocation of GLUT-4 to cell membranes. This translocation of GLUT-4 is mediated through insulin-independent phosphorylation and activation of AMPK [19-19]. Therefore, this enzyme is considered to be an awesome pharmacological target in type 2 DM.

Metformin as an Insulin Sensitizer

Metformin lowers blood glucose concentrations in T2D without causing overt hypoglycemia, known as an insulin sensitizer leading to reduction in insulin resistance and significant decrease of plasma fasting insulin level, attributed of its positive effects on insulin receptor expression and tyrosine kinase activity, on phosphorylation of IR and IRS-1 by 100 and 90% respectively in contrast insulin reduces phosphatidylinositol 3-kinase (PI 3-kinase) activity while metformin restored PI 3-kinase activity in insulin-resistant myotubes along with basal activation of p38 but insulin did not further stimulate p38 activation in metformin treated cells. Since the effect of metformin on glucose uptake corresponded to p38 MAPK activation, suggesting the potential role p38 in glucose uptake, demonstrating the direct

insulin sensitizing action of metformin on skeletal muscle cell [20, 21].

A study of metformin treated liver cells demonstrated that the mechanism of action of metformin in liver involves IR (1 micro g/ml, metfor) tyrosine phosphorylation followed by selective IRS-2 activation, and increased glucose uptake via increased GLUT-1 in a concentration-dependent manner, effect of which is completely blocked by an IR inhibitor also basal IRS-2 mRNA, transcription was up-regulated by metformin also in the presence of insulin activated Akt, dependent on phosphoinositide-3 kinase, substantially reinforce the insulin-stimulated translocation of Glut-4 transporters from the cytosol to the membrane [22].

Metformin and Mitochondrial Enzyme

In a series of comprehensive new study, Madiraju and colleagues [23], demonstrated that infusion of metformin to the experimental rat exactly in the concentrations of therapeutic range precisely reduced endogenous glucose production lowering the plasma glucose levels hence raising plasma lactate and glycerol levels, without changing hepatic gluconeogenic gene expression or cellular energy charge.^[23] Furthermore, the results compellingly documented that metformin selectively inhibits the mitochondrial isoform of glycerophosphate dehydrogenase, an enzyme that catalyzes the conversion of glycerophosphate to dihydroxyacetone phosphate (DHAP), thereby transferring a pair of electrons to the electron transport chain causing a reduction in cytosolic DHAP and a rise in the cytosolic NADH-NAD ratio, decreasing the conversion of lactate to pyruvate and diminishing the use of glycerol and lactate as gluconeogenic precursors to glucose, restraining hepatic gluconeogenesis, hence building up of glycerol and lactate levels in plasma. Long-term metformin dosing reproduces these reciprocal changes in the redox state, while increasing cytosolic redox and decreasing mitochondrial redox states, thus mitochondria becomes oxidized relative to cytoplasm^[23] also study demonstrated antisense oligonucleotide knockdown of hepatic mitochondrial glycerophosphate dehydrogenase in rats resulted in a phenotype similar to chronic metformin treatment, and abrogated metformin-mediated increases in cytosolic redox state, These findings were copied in whole-body mitochondrial glycerophosphate dehydrogenase knockout mice, the results of which are very much important for the implication in inhibition of endogenous glucose production to understand the mechanism of metformin's blood glucose lowering effects and provide a new therapeutic target for type 2 diabetes [23, 24].

Metformin and Irisin

Irisin is a novel myocyte secreted hormone supposed to be ideal therapeutic target of metformin independent of AMPK activated pathway in type 2 DM as a study showed irisin treatment reduced body weight and blood glucose in metformin treated db/db mice,^[24] exhibiting metformin effect to upregulate irisin precursor FNDC5 mRNA/protein expression in muscle and enhance irisin release independent from AMPK signaling pathway suggesting novel mechanism of metformin.

Metformin as a Glucagon Antagonist

The liver is an essential organ that store glycogen and produce glucose later to the brain during fasting especially by the effect of hormone glucagon, the inability of insulin to suppress hepatic glucose output is a major etiological factor in the hyperglycemia said to be presented with insulin resistance or type 2 diabetes mellitus (T2DM) [25, 26], metformin, the most frequently prescribed drug for T2DM, suggested mechanism was it reduces glucose synthesis via activation of the enzyme AMP-activated protein kinase (AMPK) has recently been seriously challenged in a mutated experiment [27], and therefore novel mechanism by which metformin antagonizes the action of glucagon is put forward in which fasting glucose levels is reduced by inhibiting adenylate cyclase, consequently elevating intracellular AMP thus reducing levels of cyclic AMP and protein kinases A (PKA) activity, abrogate phosphorylation of critical protein targets of PKA, and block glucagon-dependent glucose output therefore inhibition of liver glucose production, from hepatocytes, the independent of AMPK, suggesting new approach to the development of antidiabetic drugs [28, 29].

Metformin Inhibit Glucagon Signaling

A plausible molecular mechanism of action now emerges from recent breakthroughs that place metformin to restrain energy homeostasis and shown to induce a mild and transient inhibition of the mitochondrial respiratory chain complex 1 that decrease hepatic energy states and activates the AMP-activated protein kinase (AMPK), a cellular metabolic calibrator (sensor), and provides a generally accepted mechanism for metformin action on hepatic gluconeogenic program [30, 31]. However, this mode of action by metformin has recently been challenged by mutational experiments, evidence showed that metformin-induced inhibition of hepatic glucose output is mediated by reducing

cellular energy charge rather than direct inhibition of gluconeogenic gene expression. Furthermore, recent data support a novel mechanism of action of metformin involving antagonism of glucagon signaling pathways by inducing the accumulation of AMP, which inhibits adenylate cyclase and reduced levels of cAMP. Since both glycogenolysis and gluconeogenesis are controlled during the fasting state in part by the hormone glucagon, whose abnormal secretion in T2DM is a major factor in the pathophysiology of hyperglycemia, metformin produces its effects by inhibiting glucagon signaling pathways [32-37], the hepatocyte plasma membrane receptor get signal from the hormone that leads to activation of adenylyl cyclase (AC), generating the second messenger cyclic AMP (cAMP), and stimulating protein kinase A (PKA), thus phosphorylates target proteins that work in concert to increase hepatic glucose output [38], endogenous P site ligand (suggested to be AMP) molecules containing an adenine moiety binds to the 'P-site' of adenylyl cyclase and inhibit its activity [39]. Though the, the physiological or pharmacological significance of this regulatory event has not previously been observed [40-42], therapeutic levels of metformin induce a mild energetic stress in hepatocytes, resulting in an increase in AMP concentration to levels capable of directly inhibiting adenylyl cyclase. Different studies suggest that the P site of adenylate cyclase might represent a novel target for the development of therapeutics for the treatment of insulin resistance and T2DM [43-48].

CONCLUSION

Metformin is the seminal therapy for diabetes mellitus since many years; the clear mechanistic of action remained ill-defined. However, proposed main effect of this drug is to decrease hepatic glucose production through a mild inhibition of the mitochondrial respiratory-chain complex 1 that results into transient decrease in cellular energy status promoting activation of AMP (energetic sensor) also known as AMPK. Thus, activated AMPK is believed to promote transcriptional inhibition of hepatic gluconeogenic gene and hence gluconeogenesis. Inhibition of hepatic glucose production by metformin is preserved in liver-specific AMPK knockout mice strongly suggest that other mechanism(s) are involved, therefore the decrease in hepatic energy status following inhibition of the respiratory-chain complex 1 by metformin is probably the central explanation for the acute reduction of hepatic gluconeogenesis by the drug. Additionally, AMPK-dependent mechanisms linked to the hepatic lipid metabolism are also proposed, notably for explaining its beneficial effect on insulin

resistance, leading to the normalization of blood glucose levels. The knowledge gained from dissecting the principal mechanisms of metformin's novel mode of action can help us to develop new therapeutic drugs for the treatment of this dreadful disease, some other evidence explains opposing the action of glucagon, suggesting the inhibition of mitochondrial complex I results in defective cAMP and protein kinase A signaling in response to glucagon. Also stimulation of 5'-AMP-activated protein kinase, although dispensable for the glucose-lowering effect of metformin, confers insulin sensitivity, mainly by modulating lipid metabolism therefore understanding of the antigluconeogenic action of metformin in the liver and the implications of new discoveries of metformin targets for the treatment of diabetes mellitus will be beneficial to the T2DM patient.

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