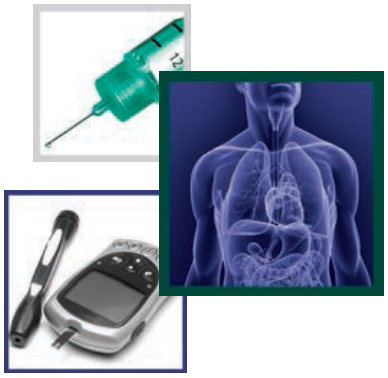
REVIEW ARTICLE

Diabetes Management



Metformin-carbonic anhydrase interaction facilitate lactate accumulation in type 2 diabetes

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ABSTRACT

Metformin has emerged as the most widely prescribed antidiabetic medication for the management of type 2 diabetes. Among the widely accepted mode of its action, is reduction of hepatic glucose production. The risk of lactic acidosis is common with metformin usage. Recent data revealed that Metformin, in addition to its glucose reduction action, might be responsible for specifically inducing lactic acidosis. The present review can help us explore the possible mechanism by which this process occurs in order to develop means of avoiding lactic acidosis. Metformin exerts its action, by inhibiting hepatic gluconeogenesis. Carbonic anhydrase a ubiquitous Zinc metalloenzyme has been found to not only facilitate in/out flux of lactate through monocarboxylate transporters across cells, but also to be responsible for the provision of bicarbonate for the first step of hepatic gluconeogenesis. Thus inhibition of carbonic anhydrase will results in defective provision of bicarbonate for hepatic glucose production and hence low blood glucose. Metformin decreases hepatic lactate uptake, through yet to be fully explained mechanism, however, these effects require further investigation. Here, the role of carbonic anhydrase in lactate transport and the rise in blood lactate level upon carbonic anhydrase inhibition has been discussed. New understanding of the carbonic anhydrase inhibition action of metformin in the liver and the implications of carbonic anhydrase inhibition has been discussed and will be important in design and development of novel antidiabetic drugs. Google scholar database search was performed using the following terms: "metformin", "gluconeogenesis", "carbonic anhydrase", "lactate". There was no restriction on the year the paper was published. Only manuscripts written in English were considered.

Introduction

The major strategies employed for the treatment of type 2 diabetes is regulating circulating blood glucose by; stimulating pancreatic β-cells to secret more insulin, increasing insulin receptor sensitivity, inhibiting hepatic glucose production and also enhancing glucose uptake by glucose utilizing tissues. Many of the oral antidiabetics' drugs decrease circulating blood sugar mainly by stimulating insulin release from the pancreatic β-cells. The present review mainly focuses on metformin in the treatment of type 2 diabetes mellitus [1-3].

Metformin is widely used to treat hyperglycemia in individuals with type 2 diabetes. It has been proposed to mediate its action on hepatic gluconeogenesis, mainly by suppressing hepatic glucose production.

Since the introduction of metformin in the 50s' the exact mechanism of its action remains to be fully elucidated. It was reported that the biguanide metformin has been in use for its

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glucose-lowering effect in Europe since 1957 and in USA since 1995 [4]. Yet despite being the most frequently prescribed antidiabetic medication worldwide, its mechanism of action remained to be fully explained. But it is believed to reduce hyperglycemia through a number of known mechanisms [2-5].

Several mechanisms have been postulated to explain the mechanism of metformin action, such as its pleiotropic actions of metformin which is associated with the activation of AMP-activated protein kinase (AMPK) [6]. It's ability to enhance insulin-stimulated glucose uptake in a variety of tissues, including adipose tissue, skeletal muscle and smooth muscle [7-9].

Another study that increases our understanding of the mechanism of metformin action is the specific inhibition of mitochondrial respiratory chain complex 1 by metformin [7,8] in both perfused livers and isolated hepatocytes from rodents and also in pancreatic beta cells [10], endothelial cells [10], neurons [11] and skeletal muscle [12]. It was reported [7,8,13,14] that mitochondrion is the primary target of metformin action within the cell, where metformin transiently inhib–its complex I of the mitochondrial electron transport chain.

Recently another mechanism of metformin action was shown in a series of *in vitro* and *in vivo* experiments that it opposes glucagon signaling, reduces hepatic glucose output and decreased production of cyclic AMP [15]. It was also reported that the critical factor underlying the effects of metformin on the regulation of hepatic glucose output is a reduction in hepatic energy status [16], but not AMPK activation. Another mechanism of metformin action that is widely accepted is the inhibition of transcription of key gluconeogenic genes in the liver [17,18]. Such as the proposal made by Ref.'s [17,19] that metformin stimulates CRTC2 phosphorylation in response to metabolic signals such as energy stress through the LKB1-AMPK/SIK1 pathways.

It has been postulated that metformin may cause a shift in the intracellular redox potential away from aerobic to anaerobic metabolism. The effect of which is increased lactate production especially in muscle and red blood cells. Increased anaerobic glycolysis in erythrocyte produces lactate as the end product of glycolysis. Red blood cells produce lactic acid as a by-product of the regeneration of ATP during anaerobic glycolysis but cannot use lactic acid, and approximately 1400 mmol of lactic acid are produced daily, which are buffered by 1400 mmol of HCO_{3-} to form sodium lactate. The rate of production can increase 50-fold if either glucose or glycogen is required to generate ATP in the absence of oxygen [20].

Metformin has also been reported to inhibit conversion of lactate to glucose through gluconeogenesis. High therapeutic metformin levels have been reported to reduce lactate uptake by the liver [21]. The effect is reduced lactate utilization by decreasing hepatic lactate uptake which will result in blood lactate accumulation and also suppression of hepatic gluconeogenesis. The physiopathology of Metformin Associated Lactic Acidosis (MALA) is complex and mostly unclear. However, this side effect may not be unconnected to the anti-hyperglycemic effect of metformin. The exact mechanism by which metformin causes lactic acidosis is yet to be defined, but many studies have suggested ways by which metformin causes lactic acidosis. One of such studies is that Biguanides reduce pyruvate dehydrogenase activity and mitochondrial transport of reducing agents and thus enhance anaerobic metabolism [2]. This switch to anaerobic metabolism, in the presence of reduced insulin, increases production of precursors for the Krebs cycle [22]. The inhibition of pyruvate dehydrogenase results in a decreased precursor's entry into aerobic metabolism, which, in turn, results in increased metabolism of pyruvate to lactate and increases the net lactic acid production.

Renal insufficiency has been reported to lower clearance of both lactate and metformin, increasing the risk of lactic acidosis [22]. Any state that results in tissue hypoperfusion can lead to tissue hypoxia, which also may cause lactic acidosis. Additionally renal failure, pulmonary disease, liver failure, cardiac impairment, shock states, severe dehydration, and microvascular disease can contribute to lactate accumulation. It is worth noting that some of the conditions (e.g., renal insufficiency, microvascular disease, and dehydration) develop in poorly controlled or advanced stages of diabetes. It has also been suggested that metformin would also be inappropriate for patients with chronic obstructive airway disease, ischemic heart disease, or severe infection, in which lactate production might be increased or due to decreased tissue perfusion [23,24].

However, Wetzel presented the first evidence for a facilitated transport of lactate by carbonic

anhydrase [25]. Ref. [26] shows that knock-out of the extracellular CA isoforms IV, IX and XIV in mouse muscle all lead to a reduction in lactate influx and efflux. A recent study has significantly enhanced our understanding of the role of CA in lactate transport [27,28]. It was demonstrated that lactate movement in/out of the cells is facilitated by carbonic anhydrase and bicarbonate transporters in various cells and tissue through mono-carboxylate transporters (MCT).

Association between carbonic anhydrase and hepatic glucose production

Carbonic Anhydrase (CA) is ubiquitous zinc metalloenzymes present in all life forms, it primarily catalyze the reversible hydration of CO_2 to HCO^{3-}, a reaction that occur slowly in the absence of the enzyme. Discovered, in 1933, the enzyme has been the subject of intense scientific investigation, due to their pharmacological applications in many diseases, such as diabetes, obesity, glaucoma, epilepsy and cancer where their therapeutic potential as antidiabetic [29,30], anti-obesity [31], antiglaucoma [32], anticonvulsant [33-35], and anticancer [36] have been investigated.

It was previously reported about the presence of endogenous carbonic anhydrase (CA) in rat liver [37,38]. The mammalian liver is the primary site of gluconeogenesis, where lactate (a gluconeogenic substrate) taken up by the liver is oxidized to pyruvate which is then converted to glucose by pyruvate carboxylase. It was reported that the carboxylation of pyruvate by pyruvate carboxylase (PC) occur exclusively in the mitochondria [39]. An earlier study supported the role of CA in supplying HCO^{3-} for pyruvate carboxylation in chameleons and alligators [40]. Previous studies have shown that, CA provides the HCO3- required for the initial steps in glucose synthesis, fatty acid synthesis, general amino acid synthesis, and urea synthesis [41-45]. It was later concluded that Inhibition of mitochondrial CA is responsible for decreased urea and glucose synthesis by alligators and chameleons *in vivo* [41], decreased urea synthesis by isolated perfused rat livers [44,46], decreased urea and glucose synthesis by isolated rat hepatocytes [47,48], and by isolated guinea pig hepatocytes [43,49,50].

It reported in one of the studies [51] with male rats that; diabetes results in a twofold increase in the activity of CA V. A similar finding was made in both STZ induced diabetic rats and in type 2 diabetic subjects [25,52] that diabetes result in increased erythrocyte carbonic anhydrase activity. These studies agreed with the earlier studies that reported the role of carbonic anhydrase in providing HCO^{3-} as substrate for pyruvate carboxylase reaction in hepatic glucose synthesis. In the same studies they also observed an increase in lactic acid concentration; they suggested that the increased lactic acid level was due to increased anaerobic oxidation of glucose in the erythrocyte and muscle cells. The lactic acid must be shuttled out of the cell to prevent intracellular lactate accumulation; lactate is shuttled out of the cell via monocarboxylate transporters facilitated by carbonic anhydrase. However, an evidence was found that supported the notion that lactate-H^+ cotransport via monocarboxylate transpoters (MCT) 1,2 and 4 is facilitated by HCO^{3-}, Carbonic anhydrase (CA) activity, Na^+ /H^+ exchange, and 1Na^+ : 2 HCO^{3-} co transport [53]. They concluded that carbonic anhydrase activity was increased in response to rising lactate level which must be shuttled out of the cell to prevent intracellular lactate accumulation which can lead to hyperlactemia and finally to lactic acidosis a condition of metabolic acidosis. Scientists showed an earlier result from their laboratory that rat kidney CA activity is increased in mild acidosis [42]. The same thing may likely happen in mild lactic acidosis. We may therefore hypothesize that the sustained increase in CA activity may be responsible for increased hepatic glucose production in diabetes, especially when it comes to greater delivery of lactate (a gluconeogenic precursor) for continued gluconeogenesis. This indicates that CA may increase hepatic glucose production in either of the two ways or both. By increasing hepatic lactate uptake or by increase provision of HCO^{3-} for the pyruvate carboxylase reaction.

Many studies have found that inhibition of carbonic anhydrase is associated with decrease in hepatic glucose production. Significant reduction in circulating blood glucose level, elevated lactate level in STZ induced diabetic rats treated with carbonic anhydrase inhibitor Acetazolamide was reported [25,26]. Similar findings have been reported that defective provision of bicarbonate, leads to hypoglycemia, elevated lactate, metabolic acidosis, hyperammonemia, and ketone bodies and excretion of carboxylase substrates [54]. Defective provision of bicarbonate comes as a result of carbonic anhydrase inhibition. It

was showed that at 0.6 μM concentration of ethoxzolamide; 50% of glucose synthesis was decreased in rats [55]. They also showed that increasing the concentrations of ethoxzolamide glucose synthesis was further decreased.

Association between metformin, carbonic anhydrase inhibition and low blood glucose

Increased hepatic glucose production has been reported in several studies to be responsible for hyperglycemia in type 2 diabetes. Clinical studies and studies in animal models have suggested that the primary function of metformin is to decrease hepatic glucose production, mainly by inhibiting gluconeogenesis [56-58]. The glucose-lowering effect of metformin can be attributed to its ability to suppress hepatic glucose production. Several mechanism have been discussed earlier in this review that tries to explain the exact mechanism of metformin action, but the exact pathway involved in the metformin inhibition of hepatic gluconeogenesis is not well explained. Mitochondria seem to be the primary targets of metformin action, still metformin can influence erythrocytes, which lack mitochondria, possibly by affecting membrane fluidity, but it was further suggested that exploration of this mechanism is needed [59].

Metformin has been reported to inhibit complex I of the respiratory chain in intact cells [60,61] but does not affect the oxidative phosphorylation machinery downstream of complex I [60]. In 1978 Dr. Peter Mitchell was awarded the Nobel Prize for Physiology and Medicine for his chemiosmotic theory, but Dr. Robert E. Forster believed that something was wrong with Dr. Mitchell's chemiosmotic theory and that a mitochondrial CA is responsible for dissipation of the proton gradient required. It has been reported that complex I inhibition by metformin interrupts mitochondrial respiration and decreases proton-driven synthesis of ATP, causing cellular energetic stress and elevation of the AMP: ATP ratio.

It was detected that mitochondrial CA V in rat liver and kidney and in the guinea pig liver and skeletal muscle [61,63]. Mitochondrial CAV was first purified from guinea pig and rat liver [49,50]. CAV may play a role in the secretion of insulin from the pancreatic B-cells was suggested[64]. It was reported that the effect of metformin on carbonic anhydrase activity both *in vitro* and *in vivo* [52]. They found out that treating STZ induced diabetic rats with metformin at 14.7mg/kg for 28 days does not only result in reduction of blood glucose but also reduction of carbonic anhydrase activity and elevation of blood lactate as well. The adverse effect of metformin is lactic acidosis. In fact severe lactic acidosis was the main reason another antidiabetic drug phenformin was withdrawn from the market. The inhibition of respiratory chain by metformin causes a shift from aerobic to anaerobic metabolism which produces lactic acid as the end product. As mentioned earlier, carbonic anhydrase is required for the transport of lactate across cells.

The implication of CA inhibition will be impairment of the aerobic oxidation of glucose which the body responds by shifting to anaerobic oxidation, which produces lactic acid as the end product. Continued anaerobic oxidation further produces more lactic acid that overwhelms the body's capacity to clear it, because the CA that is supposed to facilitate in the lactate uptake is itself inhibited, therefore lactate accumulate in the blood leading to lactic acidosis. The decrease in hepatic glucose output by metformin may be explained interms of reduced delivery of gluconeogenic substrate (lactate) brought about by CA inhibition which can no longer maintain continues supply of the substrate or due to defective provision of HCO^{3-} needed by pyruvate carboxylase for continued hepatic glucose production.

The observation that the activity of CA is significantly increased in both diabetic rats and type 2 diabetic subjects indicate the importance of CA in the provision of HCO3- for pyruvate carboxylase reaction and in blood lactate clearance by facilitating hepatic lactate uptake for greater delivery of lactate for gluconeogenesis. If the activity is not needed, then there would be no need for the increase in the enzyme activity. However, inhibition of carbonic anhydrase activity was shown to result in reduction of hepatic glucose production and also elevation of lactate concentration. Therefore Metformin may cause lactic acidosis in by inhibiting carbonic anhydrase [65-73].

Conclusion

Many studies have linked carbonic anhydrase inhibition with reduction in hepatic glucose output and blood lactate elevation. Inhibition of carbonic anhydrase may disrupt the energy cycle by preventing hepatic lactate uptake

leading to reduced glucose production and finally blood lactate accumulation. This review suggests that the effect of metformin on carbonic anhydrase can be relevant in reducing hepatic glucose production. A better understanding of the linkage and the molecular mechanisms of metformin action on carbonic anhydrase will help us better understand the causes and possible treatment of lactic acidosis in type 2 diabetes.

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