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REVIEW ARTICLE

Metformin beyond Dysglycemia

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Introduction

Metformin, an oral antidiabetic drug belongs to the biguanide class of drugs. It is one of the most widely used drugs for treatment of diabetes and is one of only two oral antidiabetic drugs on the World Health Organization (WHO) list of essential medicines. The antidiabetic effect of metformin was first reported by Jean Sterne, a French physician in 1957. The popularity of metformin gained momentum when it was found to be an effective alternative to the only available treatment then, insulin. Further studies done in this aspect showed that metformin was as promising as Sterne's first report showed. More than 50 years after its discovery, metformin has stood the test of time and its multifaceted therapeutic effect has broadened its scope in the treatment of not only type 2 diabetes, but also diseases like polycystic ovarian syndrome, gestational diabetes mellitus, prediabetes, non-alcoholic fatty liver disease and cancer. This review attempts to explore the potential value of this valuable drug in these diseases and study its role beyond control of hyperglycemia.

Metformin in Diabetes

The molecular mechanisms underlying the antidiabetic actions of metformin is a matter of debate. The antihyperglycaemic action of biguanides is mainly due to the reduced glucose output owing to inhibition of liver gluconeogenesis and possibly to a lesser extent, increased insulin-mediated glucose uptake in the skeletal muscle. Metformin also slightly delays the glucose absorption process in the gastrointestinal tract. It increases the activity of the insulin receptor and of insulin receptor substrate 2 (IRS-2) and enhances glucose uptake via increased translocation of glucose transporters, such as GLUT-1 to the plasma membrane. As a result, metformin enhances the insulin-mediated suppression of gluconeogenesis. Furthermore, and possibly of greater importance, metformin opposes the gluconeogenic action of the peptide hormone glucagon. The net effect of the interactions is that metformin inhibits gluconeogenic enzymes and stimulates glycolysis by altering the activity of multiple enzymes in these pathways. The uptake of gluconeogenic substrates, such as alanine and lactate is reduced in the presence of metformin, possibly owing to depolarization of the hepatocyte membrane through metformin-stimulated Cl^- efflux.[1]

Metformin improves insulin sensitivity and insulin-mediated glucose uptake in skeletal muscle through an increase in the tyrosine kinase activity of the insulin receptor and through enhanced activity and translocation of glucose transporters, such as GLUT-4 to the plasma membrane.[2]

Studies on autoptic pancreatic tissue have revealed that the islets in type 2 diabetes may have decreased beta cell mass as well as functional defects. A recent study[3] reported data on the characteristics of pancreatic islets isolated from type 2 diabeticorgan donors, in which reduced insulin content, decreased amount of mature insulin granules, impaired glucose-induced insulin secretion, reduced insulin mRNA expression, and increased apoptosis with enhanced activity of caspase-3 and -8 were found. These alterations were associated with evidence of increased oxidative stress. It was found in this study that metformin can reverse most of the alterations found in type 2 diabetes

islets and it was proposed to be due to improved islet redox balance. This finding is supported by studies that have shown that metformin can enter the mitochondria, accumulate within these organelles, and inhibit complex 1 of the respiratory chain[4]. Therefore, when isletcells are exposed to the drug, a lower amount of reactive oxygen species of mitochondrial origin is likely to be produced, which restores a sort of vicious circle, leading to reduced oxidative stress.

The effectiveness of metformin in the treatment of type 2 diabetes mellitus has been proven by various studies and it is advocated as the first line treatment for newly diagnosed type 2 diabetics by the American Diabetic Association (ADA) because of its low risk of hypoglycemia, the likelihood of modest weight loss, the reasonable durability of its antihyperglycemic effects and its long-term general and cardiovascular safety record. There are reservations regarding use of metformin in the presence of chronic kidney disease and the guidelines endorsed by the various bodies (ADA, AACE, UK-NICE guidelines, US guidelines) recommend different eGFR cut-offs for metformin use.

A Swedish study in which 51 675 men and women with type 2 diabetes, registered in the Swedish National Diabetes Register and on continuous glucose-lowering treatment with oral hypoglycaemic agents (OHAs) or insulin were studied, found that metformin showed lower risk than insulin for cardiovascular disease (CVD) and all-cause mortality and slightly lower risk for all-cause mortality compared with other OHAs. Patients with renal impairment showed no increased risk of CVD, all-cause mortality or acidosis/serious infection in this study.[5]The question whether current contraindications are too restrictive still remains and standardisation of prescribing advice and consensus on use are required.

Metformin in Prediabetes

The Diabetes Prevention Program (DPP) was a 27-center randomized clinical trial to determine whether lifestyle intervention or pharmacological therapy (metformin) would prevent or delay the onset of diabetes in individuals with impaired glucose tolerance (IGT) who are at high risk for the disease. It was found that both lifestyle intervention and metformin were effective in decreasing the incidence of diabetes. Lifestyle intervention decreased the incidence of type 2 diabetes by 58% compared with 31% in the metformin-treated group.[6]Although metformin was less effective than lifestyle modification in the DPP and the U.S. Diabetes Prevention Program Outcomes Study (DPPOS), it may be cost-saving over a 10-year period.[7] It was as effective as lifestyle modification in participants with a BMI e”35 kg/m^2.The ADA recommends consideration of metformin use in those with impaired glucose tolerance, impaired fasting glucose or an A1C 5.7–6.4%, especially for those with BMI >35 kg/m^2, aged <60 years and women with prior Gestational Diabetes Mellitus (GDM).

Metformin in Polycystic Ovarian Syndrome (PCOS)

PCOS affects 5 to 10% of women of child bearing age and is the most common cause of an ovulatory infertility in developed countries. It is characterized by menstrual irregularities and signs of androgen excess such as hirsutism, acne and alopecia. Insulin resistance plays a central pathogenic role in PCOS and can explain most of the features of the syndrome as well as the predisposition to develop type 2 diabetes, metabolic syndrome and cardiovascular disease.

As the ovaries of women with polycystic ovary syndrome remain sensitive to insulin in contrast to tissues like muscle and fat, the hyperinsulinemic environment favors the ovarian production of excess androgen by activating its homologous receptor. Hyperinsulinemia inhibits the hepatic production of sex hormone–binding globulin, thereby increasing circulating free testosterone levels. Hyperinsulinemia also inhibits the hepatic secretion of the IGF binding protein (IGFBP)-1, leading to increased bioactivity of IGF-Iand -II, two important regulators of ovarian follicular maturation and steroidogenesis. The IGF-I and -II systemic increase augments ovarian androgen production from the cacells by acting on IGF-I receptors.[8]Finally, insulin impedes ovulation, either by directly affecting follicular development or by indirectly increasing intraovarian androgen levels or altering gonadotropin secretion.

Further evidence to the pathogenic role of hyperinsulinemia is provided by the fact that measures to lower the insulin levels result in increased frequency of ovulation or menses and may also lead to reduced serum testosterone levels. Metformin effect on hyperandrogenism has been explained by the reduced ovarian and adrenal secretion of androgens, a reduced pituitary secretion of LH and an increased liver SHBG production.[9]

Studies done in PCOS have confirmed the ability of metformin to reduce insulin and androgen levels. This translates into an increased frequency of ovulation and improved menstrual cyclicity, apart from a reduction

of the hyperandrogenic features. A meta-analysis by Lord et al,[10] in which 13 trials were included and > 500 women with PCOS were studied, revealed that metformin is effective in achieving ovulation in women with polycystic ovary syndrome with odds ratios of 3.88 (95% confidence interval 2.25 to 6.69) for metformin compared with placebo and 4.41 (2.37 to 8.22) for metformin and clomifene compared with clomifene alone. An analysis of pregnancy rates shows a significant treatment effect for metformin and clomifene (odds ratio 4.40, 1.96 to 9.85). Metformin was also found to reduce the fasting insulin concentrations, blood pressure and low density lipoprotein cholesterol.

In order to evaluate the efficacy of metformin in the treatment of PCOS, various head to head comparisons have been done with clomiphene citrate (CC) with conflicting results. In the study by Palomba*et al.*,[11] the cumulative ovulation rate was similar in women treated with CC or metformin(62.0 *vs.* 84.0%, respectively), whereas the pregnancy rate was significantly higher (32.0 *vs.* 62.0%, respectively) in women treated with metformin compared with those treated with CC although this did not translate into significant number of live births (18.0 *vs.* 5.2%, respectively) in women treated with CC *vs.* metformin, although a trend favoring the metformin group was present. Another study by Legro*et al.* [12] reported that CC was superior to metformin in increasing cumulative ovulation (75.1 *vs.* 55.3%, respectively), pregnancy(29.7 *vs.* 12.0%, respectively) and live-birth (22.5 *vs.*7.2%, respectively) rates. Lastly, in the study by Zain *et al.*[13], the cumulative ovulation rate was significantly higher in CC than metformin (59.0 *vs.* 23.7%, respectively), whereas there were no statistical differences in the rates of pregnancies (15.4 *vs.* 7.9%, respectively) and live births(15.4 *vs.* 7.9%, respectively). The conflicting results of these studies could be due to the heterogeneity of the population studied. Nevertheless, metformin is still considered one of the first line drugs for the treatment of the polycystic ovary syndrome, although it is not yet approved by the Food and Drug Administration for this purpose. The safety data for metformin in pregnancy is reassuring in that multiple studies[14] have repeatedly confirmed that there is no evidence of an increased risk for major malformations.

Metformin and Cancer

Recent studies suggest that metformin may reduce the risk of cancer, but its mode of action in cancer remains unclear. Various experimental studies have been done in this regard. One such study[15] investigated the effect of metformin on human prostate cancer cell proliferation *in vitro* and *in vivo*. The results showed that metformin affects the expression and the phosphorylation of key proteins of the cell cycle and leads to an arrest in G_0/G_1 in human prostate cancer cells which is correlated with a decrease of expression of cyclin D1 and phosphorylation of pRb. The cyclin D1 gene is amplified and/or over expressed in several types of human cancer. Furthermore, increased expression of cyclin D1 enhanced cell growth and tumorigenicity. This study also revealed that apoptosis is not implicated in the antiproliferative effects of metformin. Although the effects of metformin have been essentially attributed to its ability to activate the AMPK pathway, this series of experiments demonstrated that at the protein and cellular level, the AMPK pathway plays no role in the effect of metformin on cell cycle.

Another study [16]reported that the anti neoplastic activity of metformin was by down-regulation of mammalian Target Of Rapamycin (mTOR) signaling through activation of AMP-activated protein kinase (AMPK). mTOR is a serine–threonine protein kinase which is up-regulated in many cancer cells as a result of genetic alterations or aberrant activation of the components of PI3-k/Akt pathway, contributing to dysregulation of cell proliferation, growth, differentiation and survival. In breast cancer cells this occurs through the stimulation of epidermal growth factor receptor (EGFR), the estrogen receptor (ER), as well as the insulin and IGF1R, which in turn enhance cell proliferation and cancer progression. AMPK is an energy-sensing/signaling intracellular protein which is activated in response to cellular stresses that deplete cellular energy levels and increase the AMP/ATP ratio.

The activity of this protein ensures that cell division, which is a highly energy-consuming process, only proceeds if cells have sufficient metabolic resources to support cell proliferation. AMPK activation by metformin is not only necessary for inhibition of gluconeogenesis in hepatocytes and reduction of Acetyl-CoA carboxylase (ACC) activity and hence fatty acid oxidation, but also for its growth-inhibitory effect in epithelial cells, an effect associated with decreased mTOR activation. Several studies have now demonstrated that activation of AMPK suppresses mTOR signaling induced by growth factors and amino acids.

There is yet another proposed mode of action of metformin through which it may exert its anti-cancer effect. Fatty acid synthesis is markedly increased *de*

novo in many cancer cells including breast cancer, as a result of high expression of fatty acid synthase (FAS), a key enzyme for fatty acid synthesis. High levels of FAS appear to be associated with the malignant phenotype of breast and ovarian cancers, and inhibition of FAS suppresses cancer proliferation and induces cell death through apoptosis. FAS expression has also been correlated with Her-2 over expression, and FAS inhibition repressed Her2 expression at the transcriptional level. Phosphorylation and activation of AMPK by metformin leads to suppression of FAS gene expression and inactivation of ACC, and this causes reduction in lipogenesis and synthesis of the ACC product malonyl-CoA, resulting in increased fatty acid oxidation.

Metformin and Gestational Diabetes Mellitus

The Endocrine Society recommends use of metformin therapy only for those women with GDM who do not have satisfactory glycemic control despite medical nutrition therapy, refuse or cannot use insulin or glyburide and are not in the first trimester. Breast feeding women with overt diabetes successfully using metformin during pregnancy should continue to use metformin according to these recommendations. Metformin is a more acceptable form of treatment than insulin in women with gestational diabetes although its use in pregnancy is controversial.

The Metformin in Gestational DiabetesTrial[17] studied 751 women with gestational diabetes mellitus at 20 to 33 weeks of gestation who were subjected to open treatment with metformin (with supplemental insulin if required) or insulin. There was no significant difference in the incidence of neonatal complications in the groups. Severe hypoglycemia (glucose level <1.6 mmol per liter) was less common in the metformin group, but preterm birth (before 37 weeks of gestation) was more common in the metformin group although the increased rate of preterm birth was not associated with higher rates of other complications. Glucose targets were reached sooner in the metformin group and the rates of maternal hypertensive complications did not differ significantly between the two groups.

Although uncertainties exist regarding the effect on off springs, data from a study,[18] in which 126 infants of women with polycysticovarian syndrome who were treated with metformin were reassessed at 18 months of age, have provided preliminary reassurance of a lack of effect of metformin on growth and on motor and social development. Other studies[19-24] on metformin in pregnancy have also shown favorable outcomes except for one small, retrospective cohort study[25] that showed increased rates of perinatal loss and preeclampsiaas compared with insulin treatment. Nevertheless, metformin has emerged as a safe and acceptable drug in treatment of diabetes in pregnancy, alone or in combination with insulin.

Metformin and Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD has been strongly linked to diabetes and insulin resistance and it can also predict the development of diabetes. A higher incidence of vascular complications of diabetes have been noted in those with NAFLD .[26]Studies have shown that metformin improves the insulin resistance associated with NAFLD and probably the liver enzymes as well but did not show consistent results in improving the liver histology.[27-31] The TONIC trial[32] in which a head to head comparison of metformin and vitamin E was done in biopsy proven NAFLD in 173 children (age 8–17 years) without diabetes demonstrated significant improvement in hepatocellular ballooning only in the metformin group compared with placebo. No significant differences were found between metformin and placebo when examining other histological features or the primary outcome of sustained improvement in ALT over time. Despite inconsistent results on NAFLD itself, metformin use may be beneficial in minimizing the increased risk of hepatocellular carcinoma (HCC). The inhibitory effect of metformin on cancer cell growth has been discussed earlier. Case-control studies have shown that the Odds ratio of developing HCC in patients with diabetes treated with metformin reduces to 0.3 when compared with those without this therapy .[33,34]

Metformin and Cardiovascular Disease

Various studies have examined the effects of metformin on cardiovascular disease, both in diabetics as well as non-diabetics. In patients with type 2 DM, the UK Prospective Diabetes Study (UKPDS) showed that metformin may be cardioprotective in that metformin significantly decreased all-cause mortality and stroke end-points.[35]A recent experimental study[36] demonstrated that a very low dose of metformin exerteda cardioprotective effect in a nondiabetic murinemodel of myocardial ischemia-reperfusion injury, improving AMPK activation, already activated by myocardialischemia as an endogenous protective signaling mechanism, and increasing

endothelial nitric oxide synthase phosphorylation. The GIPS III trial [37]was a double-blind, placebo-controlled study conducted among 380 non diabetic patients who underwent primary percutaneous coronary intervention (PCI) for ST segment elevation myocardial infarction (STEMI) to evaluate the effect of metformin treatment on preservation of left ventricular function after STEMI. The trial showed that the use of metformin compared with placebo did not result in improved LVEF after 4 months in these patients.

A meta-analysis of randomized clinical trials by Lamannaet al.[38]to assess the effects of metformin on the incidence of cardiovascular events and mortality showed that overall metformin was not associated with significant harm or benefit on cardiovascular events. In trials versus placebo/no therapy, a significant benefit was observed but not in active-comparator trials. Although metformin monotherapy was shown to be associated with improved survival, concomitant use with sulphonylureas was associated with reduced survival. AMPK plays a key role in the regulation of cellular lipid metabolism increasing the rate of fatty acidoxidation (FAO),[39,40] and metformin could act as lipid lowering agent activating AMPK and thus increasing FAO. Metformin treatment was shown to significantly increase HDL-cholesterol and to significantly reduce LDL-cholesterol and triglycerides compared with placebo or no treatment.[41]

In a study involving an unselected population of overweight and obese patients, metformin reduced the incidence of dyslipidemia significantly more than diet.[42]In patients with type 2 diabetes, plasminogen activation inhibitor (PAI-1),a glycoprotein whose main role is in the inhibition of plasm information during plasminogen activation and fibrinolysishas been found to be increased and also related to insulin resistance. Metformin has been demonstrated to reduce the levels of PAI-1 in type 2 diabetes, thus contributing to a decrease in cardiovascular morbidity.[43]Insulin resistance is associated with a state of chronic inflammation and metformin has been shown to have a beneficial effect on the inflammatory markers by dose-dependently inhibitingIL-1â induced release of the proinflammatory cytokinesIL-6 and IL-8 in endothelial cells, human vascular smooth muscle cells, and macrophages.[43] It also reduces the level of plasma migration inhibitor which are elevated in the obese and contribute to a pro-inflammatory state.[44]All these varied effects of metformin probably contribute to its antiatherogenic action and improved cardiovascular morbidity in type 2 diabetes.

Conclusion

To conclude, the therapeutic actions of metformin encompass a wide variety of disease pathologies centering around insulin resistance and the effect of metformin goes way beyond control of hyperglycemia. It should be emphasized that metformin should be considered as an adjunct and not a replacement to the more potent treatment options, namely lifestyle modifications like diet and exercise. Although metformin is not advocated as the first line therapy for the conditions described, except type 2 diabetes, each of these therapeutic avenues remains to be explored and future studies will probably guide us in these directions.

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"Sometimes you put walls up not to keep people out, but to see who cares enough to break them down."

— Socrates