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Review Article

Metformin: Off-Label Use and Epigenetic Mechanisms Underlying its Possible Adverse Effects During Pregnancy

Noel Taboada Lugo*

Medical Doctor, Master in Sciences, Doctor in Philosophy, Clinical Geneticist, Villa Clara University of Medical Sciences, Cuba

***Corresponding Author:** Noel Taboada Lugo, Medical Doctor, Master in Sciences, Doctor in Philosophy, Clinical Geneticist, Villa Clara University of Medical Sciences, Cuba.

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Abstract

Metformin is the most commonly used medication for the treatment of Type II diabetes mellitus worldwide. Currently, the prescription of this drug in other entities is described, which may lead to repositioning or readaptation of the drug. Internationally, the use of this medication during pregnancy has increased significantly in recent decades, because it is relatively cheap, easy to administer, and is associated with clear benefits as a treatment for hyperglycemia during pregnancy. However, it is known that it has an effect on the monocarbon metabolic pathway similar to that of folic acid antagonist drugs, a micronutrient that is essential to achieve adequate levels of DNA methylation necessary during the morphogenesis process. A bibliographic review was carried out in September 2023, based on chronological and thematic criteria, both for scientific monographs and for articles published in national and international medical journals, in Spanish and English. For this purpose, the Google Scholar search engine was used and the databases were consulted: PubMed, Medline, Bireme (Scielo, Lilacs and Cochrane) with the objective of describing the association between the use of metformin and possible adverse outcomes in the product of pregnancy, detail the off-label use of this drug in other clinical entities and describe the genetic and epigenetic mechanisms that underlie the folic acid antagonism described in this. Regarding possible off label indications, researchers are studying metformin for its potential anti-aging, anti-cancer, and immunomodulation effects. This activity warrants a comprehensive review of the indications, precautions, and potential adverse effects of metformin during pregnancy due to its antifolate effect.

Keywords: *Metformin; Type II Diabetes Mellitus; Gestational Diabetes Mellitus; Polycystic Ovary Syndrome; COVID 19; Epigenetic Mechanisms; Epigenetics*

Introduction

Metformin (1,1-dimethylbiguanide dihydrochloride) is a synthetic biguanide with two guanidine molecules, which is absorbed mainly in the upper part of the small intestine slowly and incompletely and with limited oral bioavailability, between 50-60%, although it is reduced when administered with food. The usual dose for adequate long-term glycemic control is between 0.5 and 2.5 grams daily and has a short plasma half-life, between 2 and 6 hours [1,2].

It was first synthesized in 1922 and the first report of its use as an agent with a hypoglycemic effect in rabbits was published seven years later. It is the first-line drug in monotherapy for Type II diabetes mellitus after failure of glycemic control with lifestyle modifications and strict dietary treatment [1,3,4].

At the end of the 1950s it was used for the first time in the treatment of Type II diabetes mellitus and is still the most commonly used medication for the treatment of this disease worldwide, which is used by approximately 150 and 200 million people daily [1,2,5].

Metformin is considered a drug for safe use in pregnancy, with a low teratogenic risk as it is included by the United States Food and Drug Administration (FDA) in risk category B. For use in pregnant women of drugs with low risk teratogenic, as those included in FDA groups A and B, where it is not necessary to evaluate the risk-benefit for its use in pregnant women [6,7].

An important aspect in its pharmacokinetics is that, unlike insulin, it crosses the blood-placental barrier, exposing the fetus to concentrations close to those of the maternal circulation, with similar actions on carbohydrate metabolism in both [3,8].

The most common medical indication for the drug is for the treatment of obese patients with Type II diabetes mellitus. However, new applications of some drugs are sometimes discovered in what is called 'off-label' use. In this sense, the prescription of metformin has been described in other clinical entities, which can lead to what is called repositioning or readjustment of the drug, after the regulatory approval process for its new therapeutic use [9].

It is known that metformin has an effect on the monocarbon metabolic pathway similar to that of folic acid antagonist drugs, a micronutrient that is essential to achieve adequate levels of DNA methylation, necessary for the morphogenesis process, through different epigenetic mechanisms [7].

For these reasons, the author was motivated to carry out this review with the objective of describing the association between the use of metformin and possible adverse results in the product, detailing the off-label use of this drug in other clinical entities and describing the genetic and epigenetic mechanisms underlying the folic acid antagonism described in this drug.

Methods

The bibliographic review was carried out in September 2023, based on chronological and thematic criteria, both for scientific monographs and for articles published in national and international medical journals, in printed or online versions, in Spanish or English. For this purpose, the Google Scholar search engine was used and the databases were consulted: PubMed, Medline, Bireme (Scielo, Lilacs and Cochrane).

Development

Use of metformin during pregnancy and possible adverse effects on the product

Internationally, the use of metformin during pregnancy has increased significantly in recent decades, because it is relatively cheap, easy to administer, and is associated with clear benefits as a treatment for hyperglycemia during pregnancy [10].

Different studies have investigated the association between the use of metformin in pregnant women and the condition of the newborn, with different analytical designs and population studies, the results of which suggest that metformin may be associated with an increase in the relative risk of being small for gestational age and a low risk for large for gestational age and neonatal hypoglycemia after intrauterine exposure to metformin compared with exposure from insulin use [3,11].

In a population-based registry-based cohort study by Brand., *et al.* in Finland, they found no increased fetal risk of being small or large for gestational age after in utero exposure to metformin compared with those born to mothers with gestational diabetes mellitus without prior treatment with this drug. However, they did show a higher risk of low birth weight and being small for gestational age in pregnancies with gestational diabetes mellitus in which metformin was used, compared to those in which insulin was prescribed, so the researchers

suggest that when antihyperglycemic treatment is required in a pregnancy with gestational diabetes mellitus complicated by intrauterine growth restriction, the use of metformin should be avoided [11].

When the long-term adverse effects in the offspring of mothers treated with metformin and insulin in monotherapy or in combination of both drugs during pregnancy were investigated, no association was found between the use of metformin alone or in combination with insulin and the risk of presenting long-term obesity, hypo or hyperglycemia, diabetes or alterations in psychomotor development in their offspring, compared to those in whom insulin alone was used [3].

Although in utero fetal exposure to metformin is considered safe, since it crosses the placental barrier, there are controversies regarding safety and efficacy during its use in pregnancy, some researchers have found fetal risk to be small for gestational age or to develop obesity during childhood [2,12].

In a recent meta-analysis that included 28 studies and 3,976 participants to assess growth in the fetal, neonatal and childhood periods, it was found that neonates of mothers treated with metformin in the prenatal period weighed an average of 108 grams less at birth compared to those born to mothers treated with insulin, while between 18 and 24 months of age presented an average of 0.44 kilograms more weight than those exposed prenatally to insulin and at the age between 5 to 9 years they had a higher body mass index (on average 0.8 kg/m^2) [4].

Metformin is included in the United States FDA teratogenic risk group B, which means that "animal reproduction studies have not demonstrated a risk to the fetus and there are no adequate, well-controlled studies in pregnant women". However, no association has been described between its use in pregnant women and the presence of major congenital defects in their offspring [6,11].

At the beginning of 2022, the European Health Authorities approved the use of metformin during pregnancy and the periconceptional period. This authorization was based, in part, on a cohort study with long-term follow-up for more than eleven years of children prenatally exposed to this drug [11]. Other researchers also did not find an association between the use of metformin in pregnant women with Type II diabetes mellitus and the presence of congenital defects in their offspring [OR: 0.57 (95% CI: 0.40 - 0.82)] [12] or weight alterations, height or neurodevelopment at school age [13].

However, in a prospective nationwide study in Denmark that included more than one million newborns, in which paternal preconception consumption of metformin was recorded in 3.3% of the newborns, one or more major congenital defects were found, particularly of the genital tract in men. [OR: 1.40 (95% CI: 1.08 - 1.82)] [14].

Off-label use of metformin in other clinical entities

Off-label use is understood to mean the use of a medication outside the indications for which it was approved or for a different use in a different way. Approval for marketing depends on the drug regulatory agencies of each country.

With the approval and registration of a medicine, a technical sheet or dossier is defined where the conditions of use are specified, which are reflected in the package insert. When it is used outside of these conditions, it is called off-label use [15].

Based on the efficacy and safety records of the use of metformin in the treatment of Type II Diabetes mellitus, its use has been repositioned as part of the adjunctive therapy of different types of cancer, age-related diseases, inflammatory diseases, hyperinflammatory states and infectious processes such as Coronavirus disease (COVID 19) caused by the SARS-Cov-2 virus. Furthermore, metformin has been reported to the benefit of the cardiovascular diseases in patients [4,16,17].

Metformin acts at the cellular level by decreasing the expression of histones, particularly histone 3 (H3K4me3) in the regulatory region of the promoter of genes that are involved in the positive regulation of the cell cycle, which is why it is used as an adjuvant in the treatment. of certain types of lung cancer [18].

Through *in vitro* experiments, it was shown that metformin stopped the growth of breast tumors through the activation of cyclic adenosine monophosphate-dependent kinase (AMPK), one of the mechanisms involved in reducing blood glucose [19].

It is described that low doses of metformin at the level of mouse hepatocytes activate AMPK in lysosomes through an AMP-dependent mechanism that involves the recruitment of a complex composed of AXIN and the kinase LKB1 on the surface of lysosomes and that includes the union of the ATPase enzyme with the vacuolar H+ system, forming the v-ATPase regulatory complex [4,20].

In recent research published in 2022, the membrane activating protein Presenilin 2 (PEN), a subunit of the secretase complex, was identified as associated with metformin. At the molecular level, PEN 2 residues phenylalanine 35 (F35), glutamate 40 (E40) and tyrosine 47 (Y47) are critical for binding to metformin by interacting with the biguanide group of the molecule. Metformin bound to PEN 2 is recruited by a v-ATPase accessory protein, ATP6AP1, resulting in inhibition of the v-ATPase enzyme and activation of AMPK on the lysosomal surface without altering cellular AMP levels [4].

Metformin induces alterations in the intestinal microbiota, which also contributes to enhancing its antitumor effects. In studies carried out in mice, only metformin administered orally suppressed tumor growth unlike intraperitoneal injection of this drug [21].

A high accumulation of metformin has been identified in the intestine with concentrations between 30 and 300 times higher than in plasma or other tissues, suggesting that the intestine is an important reservoir of this drug in humans and in animal models [1,4].

A lower risk of developing cancer is also described in people with Type II diabetes mellitus who use metformin compared to other hypoglycemic agents. The first studies linking the protective effect of metformin on cancer were carried out in 2005 by Andrew Morris and other researchers at the University of Dundee in the United Kingdom [19].

Due to the pleiotropic effects in the mechanisms of action of metformin on different signaling pathways, the repositioning of its off-label use has expanded to include several pathophysiological conditions [2,4]. Metformin is an option that has emerged in the treatment of gestational diabetes [13] and polycystic ovary syndrome (PCOS) to improve anovulation and conception [2,22].

PCOS is an endocrine disorder that affects approximately 10% of all women in reproductive age. Although the use of metformin during pregnancy in women with PCOS appears to be safe, it is important to highlight that in pregnant women without this condition, prenatal ingestion of this drug has been associated with an increased risk of obesity in childhood, although it is not possible to distinguish between the effect of the medication and the underlying condition that led to its indication, such as type II diabetes mellitus and associated obesity [22-24].

Metformin decreases androgen levels in women with PCOS, but this is not clear during pregnancy, it increases insulin sensitivity, and inhibits hepatic glucose production by increasing the adenosine monophosphate/adenosine triphosphate (AMP/ATP) ratio which subsequently activation of AMPK. In addition, metformin suppresses the glucagon signal by decreasing hepatic cyclic adenosine monophosphate (cAMP) production, inhibiting glycerol conversion to glucose, and regulates nutrient sensing pathways in the placenta [22].

On the other hand, metformin decreases inflammation by causing the degradation of the hypoxia-inducible factor HIF1α, the main regulator of endothelial cell senescence during vascular aging, in addition to attenuating the cascade of proinflammatory signals mediated by macrophages and increasing the autophagy and normalize mitochondrial function, which helps alleviate inflammation processes associated with aging [25,26].

Treatment with metformin also improves mitochondrial functions in peripheral blood mononuclear cells, which is associated with an increase in AMPK phosphorylation and mitophagy, in addition to reducing the levels of reactive oxygen species and cytokines. proinflammatory TNF and interleukins IL-6, which is of interest to attenuate the cytokine storm and lung inflammation that is described during the course of SARS-Cov-2 virus infection [27-30].

Due to the immunomodulatory properties of metformin that directly or indirectly involve the regulation of the host innate immune response and the adaptive immune response, the therapeutic prescription is feasible to expand to other clinical entities in the future, resulting in more widespread use [1].

Effects of metformin on the single-carbon pathway and potential epigenetic mechanisms underlying its antifolate effect

The effects of metformin on the single-carbon pathway are similar to the antifolate effects of chemotherapeutic drugs [10].

Folate is found in different foods such as orange juice and other citrus juices, green leafy vegetables, beans, peanuts and lentils, among others, where it is present in the form of conjugated polyglutamates.

Folic acid is the synthetic form that consists in its structure of a pteridine nucleus and para-amino benzoic acid linked to one or more glutamic acid residues. Once folic acid is absorbed, it is converted, by the enzyme dihydrofolate reductase, into its biologically active form: tetrahydrofolic acid. The carbon units carried by tetrahydrofolate (THF), i.e. methyl, methylene, methenyl and formyl groups, are attached to the N5 and N10 (or both) of the pteridine ring.

Folic acid is essential for the de novo synthesis of nucleotide precursors and also has the purpose of achieving adequate levels of DNA methylation, necessary for the morphogenesis process. These two main functions of folate metabolism intersect in the reaction catalyzed by the enzyme methionine synthase, which is dependent on folate and vitamin B12; Thus, on the one hand, it produces tetrahydrofolate for the synthesis of the DNA precursor nucleotide and, at the same time, it regenerates methionine from homocysteine for cellular methylation reactions [31].

The enzyme methionine synthase reductase (MTRR) is what maintains adequate levels of methylcobalamin II, a cofactor of methionine synthase. Through the action of the enzyme methylenetetrahydrofolate reductase (MTHFR), the metabolite 5,10 methylenetetrahydrofolate (5,10 MTHF) is transformed into 5 methyltetrahydrofolate (5 MTHF) and, in turn, gives rise to THF. This cascade of reactions guarantees that methyl groups are donated, which are essential for the methylation of homocysteine, and achieves the formation of methionine and S adenosyl methionine (SAM), the largest intracellular donor of methyl groups.

In DNA synthesis, with the conversion of deoxyuridyl triphosphate (dUTP) into deoxythymidyl triphosphate (dTTP), high levels of dihydrofolate (DHF) are achieved, which is incorporated into the cycle and transformed into metabolically active THF, so that the activity of the MTHFR enzyme determines the extent to which folate derivatives are directed to one pathway or another, that is, towards DNA synthesis or cellular methylation.

There are both genetic and environmental factors that determine decreasing in serum levels of folic acid: the administration of medications that inhibit the dihydrofolate reductase enzyme, for example, chemotherapy drugs, some anticonvulsants and metformin. Other environmental factors are folic acid deficiency due to gastric surgery, intestinal malabsorption syndrome, malnutrition or, simply,

due to non-ingestion of their main food sources, while genetic factors include different polymorphisms of the MTHFR enzyme [31-33].

Decreased folic acid levels produce a decrease in S adenosyl methionine (SAM) levels, which leads to insufficient DNA methylation, which is an important epigenetic mechanism that regulates genomic programming during embryogenesis. It is clearly demonstrated that folic acid plays a crucial role in the epigenetic regulation of the embryofetal development program. In addition to hematological consequences, Maternal deficiency of this micronutrient implies the occurrence of different congenital defects in the offspring [10,33].

The first epigenetic mechanism described was precisely DNA methylation, which is catalyzed by DNA methyltransferase enzymes, which transfer methyl groups (CH3) from the SAM to the 5' carbon of the cytosines present in the sites called CpG islands. At the same time, DNA methylation, histone acetylation and methylation, as well as chromatin modifications, are the best characterized epigenetic mechanisms.

The cascade of reactions that occur in the single-carbon metabolic pathway guarantees that methyl groups are donated, essential for the methylation of homocysteine, and the formation of methionine and SAM, the largest intracellular donor of methyl groups [7,32,33].

It is known that metformin has an effect on the monocarbon metabolic pathway similar to that of folic acid antagonist drugs by inhibiting the activity of the dihydrofolate reductase enzyme and, therefore, the synthesis of the metabolically active form of folate: tetrahydrofolate [10].

In a study by Marra., *et al.* they compared genome-wide DNA methylation rates between metformin users and non-users to investigate the potential epigenetic effects of metformin exposure and concluded that metformin use may alter different epigenetic mechanisms, especially DNA methylation [3,4].

There are separate, but not redundant, cytosolic and mitochondrial metabolic pathways that generate metabolites for single-carbon metabolism that can be inhibited by metformin, which can generate methionine deprivation, hyperhomocysteinemia, and decreased de novo synthesis of purines and pyrimidines necessary for DNA replication [7].

In addition, inhibition of one-carbon metabolism decreases SAM levels and increases S adenosyl homocysteine concentrations, which could have epigenetic effects on gene expression due to the decrease in DNA and histone methylation levels [10,33].

Homocysteine is a sulfur amino acid important in the transfer of methyl groups in cellular metabolism. A part of this compound binds to Serine and forms cystathionine, however, most of it is remethylated to form methionine, a key process for the single-carbon methylation cycle.

Folate or folic acid deficiency decreases the ability to remethylate homocysteine, due to an inadequate concentration of 5-methyl-tetrahydrofolate, which leads to hyperhomocysteinemia, which can also be caused by cobalamin deficiency, since vitamin B12 is an essential cofactor in the remethylation cycle that converts Hcis to methionine; or due to deficiency of other vitamins, such as B6 and B2 [35].

Hyperhomocysteinemia induces apoptosis leading to trophoblastic dysfunction. Recent studies have found an association between maternal hyperhomocysteinemia and numerous obstetric complications such as recurrent pregnancy losses, preeclampsia, preterm birth and abruptio placentae, among others [7,36].

Vidmar [37] suggests that folic acid deficiency produces an arrest of cells in the S phase of the cell cycle, which limits the processes of cell division and proliferation that are vital during the process of organogenesis.

In an investigation, an association was found between maternal hyperhomocysteinemia and the presence of congenital heart disease in offspring in mothers with the homozygous TT genotype for the C677T genetic polymorphism of the methylene tetrahydrofolate reductase (MTFR) gene, which participates in single-carbon metabolism [38].

Although the period of early organogenesis occurs during the third to eighth week of gestation, the administration of metformin for the treatment of hyperglycemia associated with gestational diabetes mellitus occurs after this critical window of embryonic development has concluded, which could explain the fact that experimental studies have not shown the presence of congenital defects related to doses of metformin that stimulate maternal AMP-activated protein kinase, a fact supported by results from large cohort studies [39].

However, compared to those of the embryo, fetal and placental cells are more differentiated and more dependent on oxidative metabolism and mitochondrial activity. Metformin inhibits complex 1 of the respiratory chain and causes an increase in the AMP-ATP ratio that stimulates the activity of AMP-activated protein kinase, which participates in the regulation of different processes, including gene expression and synthesis of proteins; among them some that are key for proper neurological and cognitive functioning.

The epigenetic effects of metformin, as a drug capable of crossing the blood-placental barrier, could have a long-term effect if chromatin modifications are passed to daughter cells during mitosis, as epimutations [7,10].

The recommended dose of folic acid in pregnant women and women of reproductive age for the prevention of congenital defects is 400 micrograms per day. In Cuba, the usual indicated dose is one thousand micrograms (1 mg) per day; However, when there is a history of previous folate-sensitive congenital defects or consumption of folic acid antagonist medications, it is recommended to increase the dose to four thousand micrograms (4 mg) daily [31].

Final Considerations

Taking into account the considerations expressed in this article, the author considers that, together with the insertion of metformin in the pharmacological treatment of gestational diabetes mellitus, not only in order to achieve optimal glycemic goals, but also to reduce maternal-fetal morbidity and mortality, it would be advisable to associate it with the use of folic acid, both from the preconception stage in women with Type II diabetes mellitus or polycystic ovary syndrome, as well as in those pregnant women who have gestational diabetes mellitus or other conditions in which metformin is prescribed off-label.

Conclusion

Metformin is currently the first-line oral therapy for the management of Type II diabetes. Its use during pregnancy has increased significantly worldwide in recent decades. Regarding possible off label indications, researchers are studying metformin for its potential anti-aging, anti-cancer, and immunomodulation effects. This activity warrants a comprehensive review of the indications, precautions, and potential adverse effects of metformin during pregnancy due to its antifolate effect.

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