Mechanisms Involved in Metformin Action in the Treatment of Polycystic Ovary Syndrome

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Abstract: The N, N´ dimethyl-biguanide : Metformin is an antidiabetic drug that increases glucose utilization in insulin-sensitive tissues. As Polycystic Ovary Syndrome (PCOS) and diabetes share some altered parameters-such as abnormal glucose: insulin ratio, altered lipidic metabolism and insulin-resistance syndrome- the use of metformin has become increasingly accepted and widespread in the treatment of PCOS. Currently, metformin is used to induce ovulation and during early pregnancy in PCOS patients, however, a complete knowledge of the metformin action has not been achieved yet. This review describes beyond the classical reproductive action of metformin and explores other benefits of the drug. In addition, the present work discusses the molecular mechanisms involved further than the classical pathway that involves the AMP-activated protein kinase.

Keywords: Polycystic ovary syndrome (PCOS), metformin, diabetes, adolescents, pregnancy, ovulation, cardiovascular.

INTRODUCTION

Polycystic ovary syndrome (PCOS) - which is characterized by hyperandrogenemia, hirsutism, oligo- or amenorrhoea and anovulation- is one of the most common endocrinological diseases encountered in premenopausal women [1, 2]. PCOS is frequently associated with hyperinsulinaemia, insulin resistance syndrome, increased cardiovascular risk and type 2 diabetes mellitus [1-3]. Its etiology is uncertain, but current theories emphasize genetic and intrauterine origins coupled with environmental factors such as diet and altered lifestyle patterns [1].

Effective treatment of PCOS remains controversial but needs to be divided into the main requirements of the patient, depending on whether they are seeking cosmetic improvement, restoration of the menstrual function, fertility, weight loss, or amelioration of metabolic changes. Reproductive disorders as low implantation rate, enhanced spontaneous abortion and neonatal mortality rate are described in women with PCOS.

The management of PCOS is complex and includes lifestyle modification combined with dietary-induced weight loss, and administration of oral contraceptives, clomiphene citrate, gonadotropins, antiandrogens and insulin-sensitizing agents. Women with properly diagnosed and managed PCOS reduces or even reverses the reproductive and metabolic morbidities and from a reduction in the risk factors for cardiovascular disorders.

Since the association of PCOS with insulin resistance impairs the pathophysiology of the syndrome, a family of the insulin-sensitizing agents, the biguanides are currently used in the treatment of PCOS. In that context, the use of N, N´dimethylbiguanide: metformin is becoming increasingly accepted and widespread. It has bee reported that metformin restores sexual cycles and is effective in protecting early pregnancy of women with PCOS [4- 6]. In addition, the aminoguanidine-like activity of metformin allows the drug to cross-talk with other pathways (such prostaglandin pathway, the nitric oxide system) and acts as a scavenger of reactive oxygen species (ROS) [7-13]. However, metformin is being clinically used without a complete understanding of the mechanism involved. This review describes beyond the classical role of metformin as an anti-hyperglycemic drug and explores mechanisms of metformin action.

METFORMIN AND CHANGES IN THE CELLULAR ENERGY CHARGE

It has been reported that metformin activates AMP-activated protein kinase pathway (AMPK) *in vitro* and *in vivo* [14, 15]. By means of this mechanism metformin, decreases glucose production and increases fatty acid oxidation in hepatocytes [14], bovine aortic cells [16], skeletal muscle cells [16] and mouse ovarian tissue [11]. Phosphorylation of treonine (Thr^{172}) of AMPK is necessary for its activity [11, 16] which is regulated by the upstream enzyme LKB1, a recently identified AMPK kinase [17]. In those studies [11, 16, 17], it has described that metformin decreases hyperglycemia without affecting insulin secretion. However, controversial studies describe the role of AMP levels in inducing metformin to activate the AMPK pathway. It has been demonstrated that when cellular energy charge decreases, metformin activates AMPK which in turn inhibits complex I of the respiratory chain [18, 19]. However, two recent studies argue against this notion since, in those studies, metformin activated AMPK without affecting the AMP/ATP ratio [20, 21]. The common point in all those studies is the finding that the key regulatory site is the phosphorylation of the Thr^{172} site on the catalytic alpha subunit of AMPK [11, 20, 21]. On the other hand, a second AMPK kinase (AMPKK) isoform that is not AMP-dependent has been reported [17, 22, 23].

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1381-6128/09 $55.00+.00

Recently, Zou *et al* [16] established that the mechanism by which metformin activates AMPK is mediated by mitochondria-derived reactive nitrogen species. In that work, the authors found that the activation of either c-Src kinase or phosphoinositide 3-kinase (PI3K) generates a metabolite or a molecule -no identified- that in turn activates AMPK via the LKB1 complex. In addition, the polymorphism in the STK11 gene (gene that codify by the LKB1) is associated to the chance of ovulation and was described as a target for the metformin action [24].

METFORMIN AND CARDIOVASCULAR RISK

One consequence of altered lipid metabolism is the increased cardiovascular risk shown in women with PCOS. The endogenous nitric oxide synthase inhibitor asymmetric dimethyl-L-arginine (ADMA) is associated with the development of atherosclerosis and represents an independent marker for cardiovascular morbidity and mortality. In fact, women with PCOS have elevated levels of ADMA and metformin treatment decreases ADMA levels independently of body mass index (BMI) and metabolic changes [25]. The mechanisms whereby metformin improves ADMA levels in women with PCOS are not quite clear. In that work, the authors found that inflammatory markers as protein C reactive (CRP) and interleukin 6 (IL-6) were not significantly correlated with ADMA and did not change in response to metformin. Thus, the reduction in ADMA following metformin treatment does not seem to be due to this kind of anti-inflammatory effectors [25].

METFORMIN AS AN ANTI-INFLAMMATORY DRUG

Migration inhibitor factor (MIF) is a pro-inflammatory cytokine [26-28] which has been related to obesity [29]. Dandona *et al*. [29] found that mRNA expression of MIF in the mononuclear cells is elevated in the obese, consistent with a pro-inflammatory state. The authors demonstrated that the increases in MIF are related to body mass index (BMI), fatty acids concentrations and C-reactive protein (CRP). They also found that metformin suppress plasma MIF concentration and normalizes BMI, fatty acids concentrations and CRP in the obese in a clear anti-inflammatory effect of the drug.

The pro-inflammatory status associated with the polycystic ovarian pathology is related to increased levels of serum tumor necrosis factor alpha (TNF alpha) which mediates insulin resistance [30]. The molecular mechanism involves the nuclear factor-kappa B (NF-kB) since it has been described that activation of NF-kB triggers insulin resistance and inflammation in PCOS [31]. In fact, metformin exerts a direct vascular anti-inflammatory effects by inhibiting NF-kB [32] which in turn decreases ADMA levels in cultured endothelial cells [25, 33].

METFORMIN AND OXIDATIVE STRESS

Hyperglycemia triggers oxidative stress [34] and glucose intake, even in normal subjects, leads to increased reactive oxygen species (ROS) generation and inflammation mediated by an increase in NF-kB [35]. This may partly explain the increased risk factors for the diabetic patients to develop cardiovascular complications [36, 37]. As we mentioned, metformin is able to normalize plasma glucose concentration without any stimulation of insulin production [11, 16, 17]. To discuss this point we have to consider that the molecular structure of metformin confers the biguanide an amino-guanidine-like activity [38]. That activity allows the biguanide to improve liver antioxidant potential in rats fed a high-fructose diet [39], ameliorate the antioxidant status in diabetic patients [40], modulate lipid peroxidation markers as LDL and HDL cholesterol [41] and increase reduced glutathione (GSH) in blood concentration [42] and ovarian tissue from hyperandrogenized mice [11].

Metformin is also able to increase the activities of antioxidant enzymes, such as catalase and CuZn superoxide dismutase [42]. Khouri *et al* [43] have studied the scavenging capacity of metformin against reactive oxygen species like hydroxyl ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) radicals. According to their results, the authors concluded that metformin does not scavenge $O_2^{\cdot-}$ radicals nor H_2O_2 but is able to react with $OH^{\cdot}$ radicals. In addition, it seems that metformin exerts its *in vivo* antioxidant activity by different pathways other than the simple free radical scavenging action, such as the increase of antioxidant enzyme activities [7, 42, 44], the decrease of the markers of lipid peroxidation [41, 42] and the inhibition of the formation of advanced glycation end products [45, 46]. In addition, the aminoguanidine-like activity of metformin allows the biguanide to interact to the heme-group of nitric oxide synthase enzyme and thus to regulate oxidative nitrogen species [38]. The thiazolidenediones have antioxidant properties - suppressing production of ROS- [47] and anti-inflammatory effect [48] and for these reasons represent an important option in the treatment of infertility in women with PCOS [49] many times in combination with metformin.

METFORMIN AND THE ENDOTHELIAL FUNCTION

Cardiovascular disease is the leading cause of death in women; particularly those with PCOS are at a seven-fold or greater risk for myocardial infarction [50, 51]. One of the early signs of cardiovascular lesions is the endothelial injury [52]. The mechanism by which the vascular bed is affected under the influence of various metabolic and hormonal abnormalities is not clear. Several hypotheses have been formulated, and several factors seem to have a synergistic role in this process. Insulin resistance seems to play a key role in the development of endothelial damage [53]. The increased insulin levels shown in women with PCOS are associated with decreased cardiac flow [54] and extensive coronary artery disease has been lately demonstrated in women with PCOS as well [55]. In addition, hyperandrogenemia underlies the acceleration of the endothelial injury process [56]. In agreement with those findings, Diamanti-Kandarakis *et al* [57] found that both obese and non-obese women with PCOS have elevated endothelin-1 (ET-1) levels compared with the age-matched control group. These authors also demonstrated that metformin therapy for 6 months reduces ET-1 levels [58].

Anovulatory patients with PCOS have an alteration in uterine vascularization. These patients have higher pulsatility index and resistance index, two measures of blood impe-

dance inversely related to blood flow, compared to healthy controls [58]. These authors demonstrated that metformin improves those surrogate markers of endometrial receptivity. In addition, metformin reverses the formation of pro-apoptotic structures of endometrial uterus from hyperandrogenized mice [59]. Moreover, it has been recently reported that metformin reduces human cancer incidence and improves the survival of cancer patients including those with breast cancer [60]. These actions are mediated by the action of metformin in regulating the cellular cycle. Moreover, a direct effect of metformin in regulating *in vitro* the adverse effect of excess of androgens in the proliferation of T lymphocyte has been recently reported [61].

CONCLUSION

In spite of the controversial results as regards the action of metformin, it seems that this drug represents an effective treatment for the reproductive dysfunctions and for the alterations derived of polycystic ovary syndrome. A deeper knowledge of the limitations of metformin will allow the search for combined treatment with other drugs, a methodology that has been widespread in the last years.

ACKNOWLEDGEMENT

The author wants to thank the support obtained from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), PICT 949.

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Received: May 14, 2009 **Accepted: May 15, 2009**