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The role of Inositols in PCOS - Opinion Paper

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ABSTRACT

The Polycystic Ovary Syndrome (PCOS) is a complex syndrome, with hormonal and metabolic features. The role of an altered insulin action is important for the onset and clinical manifestations of PCOS. The use of insulin-sensitizing agents as metformin or inositols is one of the key elements in PCOS management. In this opinion paper, the authors discuss about the actions of inositols (MYO and DCI) in PCOS clinical management.

Keywords: PCOS; Insulin resistance; hyperinsulinemia; myoinositol; D-chiro-inositol.

INTRODUCTION

The Polycystic Ovary Syndrome (PCOS) is diagnosed on the basis of the presence of at least two⁽¹⁾ of the three following characteristics: signs and symptoms of hyperandrogenism, anovulation and polycystic morphology of ovaries detected by ultrasound test; obviously, other causes of hyperandrogenism must be excluded. Insulin resistance with compensatory hyperinsulinemia is present in a high percentage of patients affected by this syndrome. In that manner, Polycystic Ovary Syndrome can be globally considered an endocrine-metabolic syndrome.

It is well known that the increase of insulin sensitivity could improve ovulatory function and reduce serum androgen levels⁽²⁾. Therefore, the

SOMMARIO

La Sindrome dell'Ovaio Policistico (PCOS) è una sindrome complessa, con caratteristiche ormonali e metaboliche. Il ruolo della alterazione dell'azione dell'insulina è importante per lo sviluppo e le manifestazioni cliniche della PCOS. L'uso di agenti insulino-sensibilizzanti come la metformina o gli inositoli rappresenta uno degli elementi chiave nella gestione della PCOS. In questo opinion paper, gli autori discutono riguardo le azioni degli inositoli (MYO e DCI) nella gestione clinica della PCOS.

use of current available insulin-sensitizing agents becomes important in the clinical management of PCOS

Inositol stereoisomers, in particular myoinositol (MYO) and D-chiro-inositol (DCI), are chemically hexa-hydroxy-cyclohexane, and share the same raw glucose formula (C₆H₁₂O₆); MYO and DCI are the most abundant stereoisomers of the nine stereoisomers of inositol⁽³⁾. In their conjugated form, inositols are components of cell membranes and have a crucial function in membrane integrity and intracellular signaling⁽⁴⁾. Phosphatidylinositol is the precursor of phosphatidylinositol phosphate and phosphatidylinositol bisphosphate (PIP₂), which after hydrolysis, catalysed by phospholipase C (PLC), gives inositol 1,4,5 trisphosphate; the latter acts as a second messenger of membrane receptors coupled to PLC, because it is involved in the signaling mechanism of many autacoids, hormones and neurotransmitters⁽⁴⁾. Insulin signaling typically

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involves the tyrosine kinase activity of the receptor by itself which first autophosphorylates and then phosphorylates some proteins called Insulin Receptor Substrates (IRS); one of the main targets of IRS is Phosphatidyl Inositol 3 Kinase (PI3K), which produces phosphatidyl inositol 3-phosphate and is involved in the activation of Akt, a protein kinase which eventually leads to an increased translocation of the glucose transporter into the plasma membrane⁽⁵⁾. Besides, it has also been supposed that insulin can signal through Gq / 11, and thus induce the production of a second inositol glycan messenger, called INS-2⁽⁶⁾; nevertheless, the activation of Gq / 11 by the insulin receptor has not been clearly demonstrated.

A defect in insulin signaling, particularly of the PI3K / Akt axis, is associated with insulin resistance; although the molecular mechanisms by which exogenous inositol supplementation influences insulin resistance are not yet clear, we can affirm that the numerous clinical data available show an improvement effect (see below), which constitutes the conceptual basis for use of inositols in the clinical management of Polycystic Ovary Syndrome (Polycystic Ovary Syndrome, PCOS).

As mentioned above, the two main isoforms of inositol of clinical interest are MYO and DCI.

The epimerase enzyme (NAD / NADH-dependent enzyme, whose action is stimulated by insulin) converts MYO into DCI, thus maintaining the balance between the levels of these two compounds; the relative levels of MYO and DCI could vary in different tissues, with a prevalence of one over the other based on the different tissue. At the systemic level, in the intracellular environment, 90% of inositols consists in MYO.

In the presence of insulin resistance, the conversion of MYO to DCI is reduced due to a reduced activity of epimerase⁽⁷⁾. In particular, patients with PCOS, might experience dysregulation of the metabolism of inositols, characterized by an imbalance, with an excess of MYO and a deficiency of DCI, together with a reduction in the activity of the MYO / DCI epimerase.

Furthermore, several studies have largely shown that there is a linear relationship between the reduced urinary excretion of DCI and the degree of IR. In particular, studies on the concentrations of DCI in tissues demonstrated that there was a rather generalized deficiency of DCI in the tissues of subjects suffering from type 2 diabetes (including muscle tissue).

Hyperinsulinemia alters the relationship between FSH and LH, inhibiting the selection process of the dominant follicle; in addition,

insulin acts as a cofactor of LH to produce more androgenic hormones at the level of the ovarian follicle, through the stimulation of the androgen biosynthetic pathway mediated by cytochrome P450 activity (in particular, P450P17). Thus, high levels of insulin can interfere with the ovulatory mechanism. It is necessary to keep in mind, in this regard, the data of a reduction in androgen levels under treatment with DCI in women with PCOS.

THE BALANCE OF INOSITOLS

The relative concentrations of MYO and DCI are dynamic, and may differ under normal and pathological conditions. First of all, the concentrations are organ-specific. Diabetes represents a condition in which an inositol imbalance was observed, with a MYO / DCI ratio of 20.4 (type 2 diabetes) or 13.6 (type 1 diabetes), compared to 13.2 (non-diabetic relatives of patients with type 2 diabetes) and 2.5 (controls) in the urine. In samples of muscle biopsies, the concentration of MYO is increased in subjects with type 2 diabetes, and further increases after insulin administration⁽⁶⁾.

Usually, when a patient progresses from a normal condition to an impaired glucose tolerance, and then to type 2 diabetes, the urinary DCI progressively decreases. This imbalance of inositol appears to be related to insulin resistance. A similar deficiency of DCI is found in women with PCOS and with preeclampsia, which both might underlie an insulin resistance. The deficiency of DCI may be due to a defect in the conversion of MYO by the epimerase. This conversion ranges from 0.7% in the heart and 2.2% in the liver to 36% in the urine and 60.4% in the blood (data on non-diabetic rats); however, conversion is much lower in diabetic rats compared to non-diabetic animals^(6,7).

INOSITOLS: CLINICAL USE

Many studies have shown that both MYO and DCI are able to reduce insulin levels, improve ovarian function and reduce androgen levels in women with PCOS^(8,9).

MYOINOSITOL

It has been shown by many studies that MYO is able to increase the number of good quality oocytes, clinical pregnancies, and birth rates in overweight women with PCOS. In every study, MYO has been administered at a dose of 2 g for a period varying from 3 to 6 months. Its biochemical, endocrine and clinical benefits are believed to be due to its insulin

sensitizing action.

In particular, one study⁽¹⁰⁾ that used MYO with a dose of 2g + folic acid, twice a day, continuously until the end of the study duration or until a positive pregnancy test was obtained; of the 25 women with PCOS enclosed in this study, 88% (n=22) had again a first spontaneous regular menstrual cycle during the study; among these 22 women, 18 kept having a regular cycle with spontaneous ovulation. Moreover, during the study the length of the menstrual cycle improved (from 34.6 ± 5.5 to 31.7 ± 3.2 days), and there was a reduction in free testosterone and testosterone levels at the serum level; during 6 months of treatment, 10 biochemical pregnancies occurred, of which 1 ended up as a miscarriage and 1 as a biochemical abortion. Multiple pregnancies were not reported.

A study⁽⁸⁾ conducted on 50 PCOS overweight women showed that 2 g of MyO + folic acid (versus folic acid alone), administered for 12 weeks, was able to reduce the risk of ovarian hyperstimulation during protocols for ovulation induction; in particular, the levels of LH, prolactin, androstenedione, insulin and the LH / FSH ratio were significantly reduced; moreover, the sensitivity to insulin appeared to be improved, with a simultaneous reduction of the area under the insulin curve. Interestingly, the duration of ovulation induction therapy and the required FSH doses were significantly lower in the MYO treated group than in the untreated control group; even if the MYO group obtained a lower oocyte recovery, however it had a higher proportion of larger oocytes (better oocyte quality), which led to a higher pregnancy rate than the untreated group, in terms of biochemical pregnancy (15 vs 8), clinical pregnancy (10 vs 4) and births (8 vs 3), with statistically significant differences. Thus, based on these data, MYO use can improve ovarian activity.

D-CHIRO-INOSITOL

Several studies have evaluated the effect of DCI on various parameters in PCOS women.

In a historical study, Nestler et al.⁽⁹⁾ used DCI at a dose of 1200 mg per day versus placebo in 44 obese PCOS women (22 treated with DCI and 22 with placebo) from 6 to 8 weeks. In the 22 women with PCOS treated with DCI, the area under insulin curve after oral glucose loading showed a statistically significant reduction ($p = 0.07$) both with respect to the baseline and with respect to the placebo group, while glucose tolerance did not vary; free testosterone levels in the serum decreased from 1.1 ± 0.8 to 0.5 ± 0.5 ng / dl ($p = 0.006$ vs placebo);

even the total testosterone values decreased from 90 ± 47 to 61 ± 33 ng / dl ($p = 0.003$ vs placebo) and those of DHEAS from 519 ± 229 to 274 ± 9 ($p = 0.001$ vs placebo), while there was a significant increase in sex hormone binding globulin (SHBG) values ($p = 0.00$ vs placebo). The triglycerides too were significantly reduced from 184 ± 88 to 110 ± 61 mg / dl ($p = 0.002$ vs placebo). Diastolic and systolic blood pressure showed a reduction of 4 mmHg ($p < 0.001$ and $p = 0.05$, respectively, vs. changes in the placebo group). Nineteen of the 22 women taking DCI ovulated, compared to 6 out of 22 in the placebo group ($p < 0.001$).

To better understand the effects of DCI at the ovarian level, it is interesting the study by Sacchi et al. published in 2016⁽¹¹⁾, in which the effects of DCI and insulin combined with FSH and LH on key steroidogenic enzymes, such as cytochrome CYP19A1 and side-chain cleavage P450scc in primary granulosa cell cultures were evaluated. Furthermore, it was assessed whether DCI, as an insulin-sensitizer, was able to oppose the expected insulin stimulation activity at the granulosa cell level. The results of the study demonstrate how, effectively, DCI is able to reduce the gene expression of CYP19A1, P450scc and of the receptor for insulin-like growth factor-1 (IGF-1) in a dose-dependent manner; therefore, DCI appears to be able to significantly reduce the increased expression of steroidogenic enzymes due to insulin treatment in gonadotropin-stimulated granulosa cells. These in vitro data can help to further explain how DCI, used in insulin-resistant subjects, can even exert beneficial effects directly at the level of the ovary.

In another, randomized, placebo-controlled study⁽¹²⁾, the administration of DCI at a dose of 600 mg per day ($n = 10$) (vs placebo, $n = 10$) for 6-8 weeks to thin women with PCOS (BMI between 20 and 24.4 kg / m²) reduced insulin levels (in terms of significant reduction in the area under the insulin curve and blood glucose after oral glucose load) ($p = 0.03$ and $p = 0.04$, respectively), testosterone total ($p = 0.003$) and free ($p = 0.01$), also decreasing the levels of systolic ($p = 0.002$) and diastolic ($p = 0.001$), total cholesterol ($p = 0.001$) and triglycerides ($p = 0.001$) serum; a higher rate of ovulation was also reported with the use of DCI (6/10 = 60%), but with a difference that was not statistically significant compared to the placebo group (2/10 = 20%), probably due to the low sample size of this study. The authors' conclusions were that, in thin women with PCOS, DCI reduces circulating insulin, decreases androgens in the serum and improves some metabolic abnormalities (increased blood pressure and hypertriglyceridemia).

Particularly important is the significant reduction of the composite body insulin sensitivity index (ISComp), a complex parameter that describes insulin sensitivity developed by Matsuda and DeFronzo⁽¹³⁾ and described by the following formula: $ISComp = 10,000 / \text{square root of } ([\text{fasting blood glucose} \times \text{fasting insulin}] \times [\text{average glycemic values} \times \text{average insulin values during OGTT}])$; the ISI showed a reduction of 84% in the group treated with DCI ($p < 0.002$).

A 2011 review⁽¹⁴⁾, which considered studies evaluating the relationship between PCOS, DCI and IR, concluded that fewer amounts of DCI-IPG are released in women with PCOS than controls and this appears to be related with the IR and hyperinsulinemia present in these patients; the administration of DCI has beneficial effects on ovulation and on anthropometric and metabolic markers in women with PCOS through the improvement of the insulin condition; finally, the effects of metformin on the improvement of insulin function in PCOS is obtained through DCI-IPG mediators.

A 2014 study⁽¹⁵⁾ evaluated the status of oxidative stress at the level of follicular fluid and oocyte quality in women with PCOS under different ovarian stimulation protocols with gonadotropins in association or not with DCI or metformin; the use of DCI or metformin was associated with a better oxidative stress in the follicular fluid than those treated with gonadotropin alone, and a significantly higher number ($p < 0.05$) of good quality oocytes was observed in subjects treated also with DCI or metformin compared to subjects treated with gonadotropin alone.

Another study⁽¹⁶⁾ evaluated the effect of the administration of DCI at a dose of 500 mg per day in overweight / obese patients ($BMI > 26$) on different hormonal and metabolic parameters. Treatment with DCI led to a significant reduction in: LH levels, LH / FSH ratio, androstenedione, testosterone, basal insulin, glucose / fasting insulin ratio, BMI. The insulin response to the OGTT also significantly improved, in terms of AUCinsulin and maximum insulin response. These improved changes were present throughout the study group, but especially in patients with diabetic relatives. Finally, the LH response to the GnRH test was also reduced after treatment with DCI. The conclusions of this study were that the administration of DCI is effective in restoring a better insulin sensitivity and a better hormonal profile in obese women with PCOS and, in particular, in hyperinsulinemic women with PCOS and diabetic relatives.

The dosage of 1g of DCI (+400 mcg of folic acid)

administered for 6 months was also evaluated in 48 women with PCOS (17), with a significant reduction in: systolic pressure, Ferriman-Gallwey score, LH, ratio LH / FSH, total testosterone, free testosterone, androstenedione, prolactin and HOMA Index, plus an increase in SHBG and blood sugar / insulin ratio. Finally, the menstrual cycle showed a significant regularization (62.5%; $p < 0.05$).

All these data show, overall, a beneficial effect of DCI on ovary.

MYO+DCI COMBINATION

The choice to use a MYO + DCI combination should be based on clinical evidence of an actual advantage over the administration of MYO or DCI alone or of the two isomers associated with other compounds (eg, alpha-lipoic acid), but, at the moment, conclusive data are lacking in this regard. Actually, there are no data that offer a rationale for establishing a given ratio between MYO and DCI to be co-administered as a supplement. Different formulations with different MYO: DCI ratio are currently available in Italy (for example: 40: 1, or 5: 1). Furthermore, the plasma levels of inositol that have been reported in several studies vary considerably. For example, as reported in a publication of 2017 (18), Baillargeon et al.⁽¹⁹⁾, reported a MYO: DCI = 111 ratio in healthy individuals, compared to an MYO: DCI = 206 ratio in women with PCOS, that is to say an increase in circulating MYO of about double, compared to a relative decrease of 500 times reported in other studies⁽²⁰⁾ which, moreover, seem to occur in the ovaries.

On the other hand, it seems somewhat difficult to think that the same relationship between two substances, taken through an oral formulation, is actually maintained at the level of the ovary or organism, after all the steps that each substance introduced per oral route must undergo: absorption, distribution, metabolism, elimination. Just think, for example, of the variability that is reported in achieving bioavailability, expressed by evaluating the AUC of the concentration / time curve for a potent and metabolic resistant compound such as ethinyl-estradiol⁽²¹⁾.

Furthermore, pharmacokinetic data obtained with labeled phosphatidyl-inositol, after oral administration, indicate that MIO and DCI accumulate above all in the liver⁽²²⁾ and do not arrive directly at the ovarian level.

Clearly, the pharmacokinetics also influences the pharmacodynamics, whereby, in the absence of single pharmacodynamic evaluations for MYO and DCI, related to the evaluation of the individual

absorption curves obtained in the case of combined administration, it is not easy to propose a use of the combination MYO + DCI versus single isomers. On the other hand, how to differentiate the patient for whom MYO may be better than DCI? or better than the MYO + DCI combination?

At the moment we do not have enough studies to be able to make a customization of the prescription, according to criteria of precision medicine. Thus, in reality, the ratio could be less important than the absolute concentrations of the two isomers. Certainly, if the purpose of using DCI is also to overcome the deficiency of the epimerase⁽⁶⁾ and to ensure adequate amounts of this isomer at the ovarian level, then the dosage of the DCI should be greater than what is available in the current combinations, as also reported in an Opinion Paper of the Italian Society of Pharmacology⁽²³⁾, also considering the fact that 500 mg daily did not bring problems to the function of the ovary, showing instead a completely adequate efficacy and safety.

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CONCLUSIONS

Based on all the above, we can state that:

1. MYO and DCI have been studied, taken separately, in PCOS women with different characteristics: overweight / obese, with a familiarity for diabetes or not, and have shown ability to improve endocrine-metabolic status, ovulation rate and quality of oocytes.

2. As far as the combination MYO + DCI is concerned, to date, we do not have reliable clinical data demonstrating its superiority compared to the supplement of only one of the two stereoisomers. Furthermore, in the absence of data on the distribution and uptake of circulating inositols following exogenous administration in humans, any alleged "optimal relationship" of the two species in a pharmaceutical preparation (including the MYO 40: DCI 1) is arbitrary, not being supported currently from experimental data of a pharmacokinetic and pharmacodynamic nature.

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