

Provisionally accepted for publication

NARRATIVE REVIEW

Inositols administration: further insights on their biological role

Running Title: Inositols and future perspectives

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DOI: 10.36129/jog.2022.40

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Word count: 1665

Declarations

Acknowledgement: None.

Conflicts of Interest: The authors declare no conflict of interest regarding this article.

Ethical Statement: N/A

Informed Consent: N/A.

Funding: No funding was obtained for the submission of our article.

Authors contribution: GB conceptualized the article; J.F. and D.C. wrote and edited the article. Z.S., M.N.D., G.C., L.D.C., and G.G. participated in the editing revision and cured the article into its final form.

Abstract

In the last decades, inositols attracted a growing interest since the acknowledgement of their role in insulin-activated signaling pathways. Myo-inositol (MI) and D-Chiro Inositol (DCI) are the most important isoforms and their use as insulin-sensitizer in treatment of polycystic ovarian syndrome is well known. New discoveries have paved the way for a wide range of new applications. For this reason, we collected the most interesting ideas and updates regarding inositols therapy to reason on innovative uses, with a particular focus in the field of infertility. We reviewed recent literature on inositol with a particular focus in the field of ovarian function and infertility. Recent researches on inositols are focusing on different signaling pathways other than the insulin-sensitizing one, such as inflammation signaling or androgens modulation. Furthermore, MI and DCI act in different ways and dosage and ratio seem to have different effects in different organs. Indeed, the use of inositols is developing promisingly in situations other than the treatment of insulin resistance and this is opening up new perspectives for increasingly personalized and effective therapies.

Keywords: inositols; infertility; metabolism; ART; PCOS

Introduction

Inositols are a group of sugar alcohols (cyclic polyols) with a 6-carbon ring and a hydroxyl group bound to each carbon and a molecular weight of 180.16 g/mol. The epimerization of the six hydroxyl groups determines nine different stereoisomeric structures. [1].

In the cells, the inositols play both a structural and functional role: the phosphatidyl-inositols are components of the cell membrane and are involved in proliferation, fertilization, contraction, vesicle and fluid secretion; the inositol-phospho glycans are involved in glucose and lipid metabolism. [2]

In animals, the most common inositol is Myo-Inositol (MI) [3] which derives primarily from dietary sources such as fruits, cereals, nuts, and animal tissues [2]. MI can also be synthesized in the human kidney and liver starting from glucose-6-phosphate by the action of D3 myo-inositol-phosphate synthase and inositol monophosphatase-1.

DCI, is another important stereoisomeric form and can be obtained from MI by an insulin-dependent intracellular epimerase [3]. Both inositols play an important role as second messengers in insulin signaling and control glucose uptake and utilization. For this reason, concentrations of inositols are different in different organs [4]. DCI concentration is high in tissues that store glycogen, where it stimulates glucose uptake, glycolysis and glycogen synthesis. [5]. Conversely, elevated content of MI is found in organs with high glucose demand such as the heart and the brain, where it increases translocation of GLUT4 (glucose uptake transporter) on the cellular surface. [6].

In the last decades, MI and DCI attracted a growing interest since the discovery of their role in insulin-activated signaling pathways and in the physiopathology of metabolic syndrome, type II diabetes [7] and management of gestational diabetes [8].

DCI and MI are considered insulin-sensitizers compounds and their deficiency causes insulin-resistance and alteration of glucidic and lipidic profile, increasing cardiovascular risk [9]

Also hypothalamus-hypophysis-ovaries axis is affected by hyperinsulinemia: insulin stimulates LH release (thus increasing LH/FSH ratio), raises androgen production from ovarian theca cells and decreases SHBG synthesis, leading to increased free testosterone levels [10]. Overall, hyperandrogenism and high levels of LH significantly disturbs the physiological process of ovarian follicular maturation and may lead to anovulatory cycles. These alterations are typically found in PCOS, one of the major causes of infertility [7, 11].

Inositols and ovaries

The concentration of inositols in ovaries is substantially higher than in plasma, suggesting that they have specific functions in these organs [4]. In particular, MI concentration in ovaries is usually 70-100 times higher than DCI concentration and it acts as second messengers not only of insulin but also of FSH with a possible role in oocyte maturation [2] and in ovulation induction [12].

MI also seems to regulate the production of anti-mullerian hormone induced by the FSH [13], which modulates the sensitivity of follicles to the FSH.

Culturing embryos [14] in media enriched with MI ameliorates embryo quality and MI has been suggested to play a key role in oocyte fertilization [15,16]. Inositol 1,4,5 phosphate is necessary for the mechanism of intracellular calcium release in the final stage of oocyte maturation [17, 18, 19].

Inositol and PCOS

Women with PCOS have a blood deficiency of DCI and normal MI levels compared to control subjects [20]. The result is insulin-resistance and hyperinsulinemia.

Treatment with inositols have shown improvement of insulin-resistance, reaching a reduction of compensatory hyperinsulinemia and improvement of metabolic and ovulatory features in patients with PCOS.

As for the ovaries, hyperinsulinemia increases epimerase function, with enhanced transformation of MI to DCI, with a relative MI deficiency.

MI stimulates the meiotic progression of oocytes into fertilization-competent eggs and its depletion is associated with alteration in the physiological process of oocyte maturation [15]. For this reason, the so-called “DCI paradox of the ovary” means that high doses of DCI in PCOS patients are associated with poor oocyte quality [21, 22] and DCI supplementation may damage oocytes [23,24].

Supplementation of MI and DCI with a 40:1 ratio showed beneficial effects on oocyte and ovarian quality, also in assisted reproductive technology, as stated in the International Consensus Conference in Florence [25]. In later study, simultaneous supplementation of MI may raise the upper limit for DCI.

It's important to tailor the therapeutic approach in PCOS patients according to the main signs and symptoms that the patient complains from, particularly if she wants to improve ovulation or ameliorate metabolic alteration or also reduce dermatological symptoms related to hyperandrogenism. MI seems to have the most marked action on metabolic profile, whereas DCI mostly affects hyperandrogenism parameters [26]. Benefits of ameliorated regularity in menstrual cycles have no significant difference between the two inositol isoforms.

Recent studies compared the use of inositols vs oral combined contraceptive pills (OCP) in the treatment of PCOS manifestation in adolescents [27,28]. In teenagers aged 13-16 years, the treatment with MI results as effective as COC in restoring the correct menstrual pattern and in reducing the hyperandrogenic manifestations in PCOS patients. Moreover, MI can be used also in patients with relative contraindications to OCP such as obesity, smokers, diabetes, migraine with aura, and thrombophilia. For the age range 17-19 years, the OCP are often preferred to MI for contraceptive purposes. However, the combination of OCP with MI results in enhancing the anti-androgenic effect and reducing the side effects of OCP such as weight gain and alteration of cardiometabolic profile.

MI is usually preferred to other insulin sensitizing drugs such as metformin or pioglitazone because of its safety and tolerability profile [29] that allow the best compliance to treatment [30]

Another theme in PCOS patients is oncologic prevention. It is well known that unbalanced levels of sex hormones, such as testosterone, can increase the risk of developing ovarian and endometrial cancer in PCOS patients [31]. Normalization of androgen levels by insulin-sensitizers such as inositols, could decrease the risk for these malignancies [32] before an early carcinoma diagnosis [33].

Inositol therapies

In literature, a complete consensus was not reached about the inositol dosage and which inositol isoform is more active to improve symptoms and biochemical profile in PCOS [34]

DCI 1200 mg once daily for six to eight weeks in overweight and obese women with PCOS led to a reduction of testosterone level, improved metabolic parameters, decreased insulin-resistance, ameliorated systolic and diastolic blood pressure, triglycerides level, and ovulatory function [7]. The effect of MI 2 g daily plus folic acid obtained insulin sensitizers is similar to metformin in overweight women with PCOS [12, 35]. Both MI 4 g daily or DCI 1 g daily achieved a decrease in circulating androgens levels, LH and LH/FSH ratio and increase in SHBG in PCOS patients [7]. Other studies compared MI 4 g daily vs DCI 1 g daily for six months or combination of both (e.g. 550 mg MI + 13.8 mg DCI daily) in women with oligo-amenorrhea without PCOS with similar results. [26]. The combination of MI 3300

mg and DCI 84 mg daily has shown better improvement on metabolic profile than MI 4 mg daily alone [36, 37]. Future studies should investigate the “right formula” to maximize the benefits in different clinical phenotypes [38].

Some patients seem to be inositol resistant because of decreased MI intestinal absorption. For these reasons, some molecules such as alpha-lactalbumin are studied as adjuvant [39]

New perspectives

Thanks to new discoveries, the use of inositols is going beyond the PCOS treatment.

The attention on inositols is changing from the role of insulin-sensitizer in PCOS patients to a more complex fan of applications.

MI seems to be helpful in “poor responders” undergoing IVF cycles [40] and the treatment with MI and melatonin improves ovarian stimulation protocols and pregnancy outcomes in infertile women with poor oocyte quality [41,42].

Due to its effect of inhibition of aromatase, high dose of DCI can be used (alone or in combination with other aromatase inhibitors, such as letrozole) for ovulation induction in PCOS population [43] but also in non-insulin resistant anovulatory women [44].

Once pregnancy has been achieved, MI can support pregnancy improving metabolic profile and preventing gestational diabetes and its fetal consequences [45]

Also, new pathways of actions have been investigated. One of the most important is the phosphatidyl-inositol-3-kinase (PI3K) pathway associated with inflammation signaling [46]. MI exerts anti-inflammatory action by downregulating IL-6 and antioxidant action by increasing the activity of glutathione synthase [47]. “Over-oxidative status” is often observed in patients with endometriosis related infertility and these abnormalities may be linked to alteration in the glucose and lipid metabolism. For this reason, periconceptual treatment with DCI, ameliorating the metabolic profile, may reduce the over-oxidative status in patients with endometriosis and improve oocyte quality, fertilization and implantation rates [48].

Some authors also proposed that MI could also prevent OHSS reducing production of VEGF and COX2 [49]. So, its supplementation could be helpful in addition to GnRH antagonist protocol in patients at high risk of OHSS [50] and that usually need to freeze all their oocyte by vitrification system [51, 52].

Another cofactor of infertility is thyroid dysfunction. Since, TSH-signaling is mediated by phosphatidyl-inositol-3-kinase (PI3K) pathway, the depletion of MI may be associated with development of hypothyroidism and supplementation with MI was shown to improve not only TSH levels, but also to reduce antithyroid antibodies and thyroid benign nodules size [53].

Combination of MI and melatonin seems to positively affect glucose metabolism also in menopausal transition [54], in addition to other specific, peculiar and innovative treatments which are gaining popularity for menopausal sexual impairment such as local hyaluronic acid administration [55, 56]. Interestingly, even though the SARS CoV-2 pandemic influenced its application, the scientific debate is still ongoing and in favor of these innovative treatment [55,56].

Finally, since aromatase converts testosterone to estradiol, the inhibition of this enzyme by high levels of DCI can lead to increased (non-converted) levels of circulating androgens, which may play a beneficial role in several conditions, such as sexual dysfunction in both

men and women [57,58]. Nevertheless, this peculiar condition should not be considered proper when dealing with PCOS, which could be affected by such molecular approach [59]. [60]

Conclusion

Inositol has been shown to play a role in improving reproductive outcomes in infertile couples. In IVF, its supplementation in association with progesterone has been used for endometrium preparation before embryo transfer [61].

In an era of denatality caused by social and economic context, parenting may be taken into consideration when fertility is compromised. For this reason, in ART it is increasingly necessary a tailored and gender approach to the infertile couple [62, 63] as well as a correct psychological counseling [64].

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