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REVIEW

New clinical targets of D-chiro-inositol: rationale and potential applications

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ABSTRACT

Introduction: Inositols have a key role in ovarian physiology and the literature reports a wealth of studies about the major isomer, myo-inositol (MI). However, information about D-chiro-inositol (DCI) is still scarce, despite the ratio MI:DCI is tissue-specific and actively maintained by an insulin-dependent epimerase enzyme.

Areas covered: This expert opinion provides an overview of the physiological contribution of DCI in regulating steroidogenesis. DCI indeed mediates the intracellular signaling of insulin, which induces the biosynthesis of androgens. Studies on second messengers of insulin also revealed that DCI has a specific role in modulating the activity of aromatase enzyme. Specifically, recent findings demonstrated that DCI influences the enzyme gene expression, thus reducing the conversion of androgens into estrogens.

Expert opinion: Available evidence suggests that the effects of DCI administration may be similar to those of aromatase inhibitors, but without causing hypo-estrogenic states. Therefore, DCI treatments should be evaluated for either estrogen-dependent gynecological conditions or low testosterone states in male subjects.

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D-chiro-inositol; aromatase; estrogen; endometriosis; controlled ovarian hyperstimulation; male hypogonadism

1. Introduction

In the last years, the role of Inositols in gynecological endocrinology and reproduction has attracted a growing interest [1]. A very recent Expert Opinion reviewed the characteristics of the two major isomers, Myo-Inositol (MI) and D-Chiro-Inositol (DCI), discussing possible clinical applications in the field of human reproduction [2]. While MI, the most abundant isomer, has a largely established role in ovarian physiology [3], the current pieces of evidence about DCI's actions are less robust, despite all organs and tissues contain variable amounts of both isomers. A specific epimerase enzyme converts MI into DCI and maintains local MI:DCI ratios, suggesting that DCI is an essential component of cellular Inositol pool and that its concentration is actively regulated. A recent report suggests that MI:DCI ratios may play a key role for several physiological processes [4]. The epimerase has a tissue-specific activity [5], leading to well-defined MI:DCI ratios in different tissues and organs [6]; this ratio is around 40:1 in the peripheral blood [7], about 20:1 in the thecal cells and within the range 70:1–100:1 in the follicular fluid of dominant follicles [8,9]. Defective regulation of the epimerase's activity results in abnormal levels of DCI and altered MI:DCI ratios, leading to impaired steroid biosynthesis [10], associated with several pathological conditions [11]. The present review focuses on the role of DCI in steroidogenesis, in particular from a gynecological perspective, discussing the potential and limitations of DCI treatments to modulate the endogenous production of estrogens and androgens.

2. D-chiro-inositol and steroid biosynthesis

In the phosphoglycan form, DCI is a second messenger of insulin signal [12,13] and indirectly influences steroidogenesis. Nevertheless, DCI exhibits also an independent activity on androgen biosynthesis, as found by Nestler and coworkers [14]. They demonstrated that a DCI-based phosphatidyl-glycan is able to stimulate the production of testosterone in human thecal cells. The same group also reported that DCI is able to reduce the activity of the enzyme CYP19A1 [15]. Such enzyme, commonly known as aromatase, is a member of the cytochrome P-450 family and catalyzes the oxidative conversion of androgens into estrogens. Consistently, 6-weeks treatment with 2400 mg/day DCI has been found to increase testosterone levels in women with Polycystic Ovary Syndrome (PCOS) [16]. In addition, Sacchi et al. found that DCI affects the gene expression of aromatase, downregulating it in a dose-response manner [17]. These observations lately prompted comparison between the activities of DCI and those of compounds known to inhibit aromatase enzyme [18], although with substantially different mechanisms of action. Aromatase inhibitors (AIs) prevent the biosynthesis of estrogens, leading to a systemic hypoestrogenic state and to potentially negative side effects, such as the loss of bone density [19]. Conversely, DCI modulates the estrogen biosynthesis affecting the androgen-to-estrogen ratio.

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Article highlights

- D-chiro-inositol (DCI), besides being a second messenger of insulin, regulates female steroidogenesis by enhancing testosterone biosynthesis and downregulating the expression of aromatase.
- As second messenger of insulin, short-term administration of DCI may reduce androgen levels in insulin-resistant women; extended treatments result in androgen increase due to the activity on aromatase and on testosterone biosynthesis.
- Taking into account the modulatory action on aromatase, DCI may find application for estrogen-dependent gynecological diseases characterized by increased expression of the enzyme.
- Tailoring the dosage and the duration of the intervention, DCI treatment may represent an appealing therapeutic approach for increasing the androgen-to-estrogen ratio in men with late-onset hypogonadism.

This box summarizes key points contained in the article.

3. D-chiro-inositol administration: duration and patients' condition

The activity of DCI on steroidogenesis, hence, may be twofold: on the one hand, an indirect effect as a result of insulin signal boosting; on the other hand, an independent direct effect on steroid biosynthesis through downregulation of the aromatase expression and enhancement of testosterone production. Most of the available data regarding treatments with DCI derives from clinical studies on PCOS women, which presented often with associated insulin resistance. In these patients, the administration of low doses of DCI causes systemic reduction of insulin resistance [20]. As a consequence, levels of androgens decrease in the short term [21], accompanied by estrogen decrease if a hyperestrogenic condition is present. Continued administration of DCI exhibits effects on aromatase and testosterone biosynthesis, with androgen levels that begin to increase again. Elevated dosages of DCI in the same type of patients may act on both pathways at one time, even for short-term administration, leading to increased androgen levels [16]. Unfortunately, to the best of our knowledge, literature data regarding the administration of DCI to non-insulin resistant women are still scarce and do not allow to draw a firm conclusion. However, based on the available information, we would expect a little initial effect on insulin levels, followed by reduced activity of aromatase. On that basis, DCI dose and timing of administration should be carefully evaluated: in particular, dosages of DCI and treatment duration should be tailored to the patients' specific condition, taking into account patients that may potentially suffer from increased androgen levels.

4. Potential clinical applications

Based on the available evidence [18], the use of DCI in gynecological clinical practice is likely to have a rationale for all those conditions that benefit from therapeutic treatments with AIs or insulin sensitizers. Moreover, adjusting the dosage and the duration of treatment, we suggest that the administration of DCI to hypogonadal men, particularly if obese, may have beneficial effects by increasing testosterone and decreasing estradiol.

4.1. Ovulation induction

Accounting for over one-third of infertile couples, the occurrence of anovulatory menstrual cycles represents one of the leading causes for infertility [22]. Long-term anovulation generally induces decreased estrogen levels [23,24], potentially resulting in reduced bone density and cardiovascular problems. According to the World Health Organization (WHO), group II is the most common type of anovulatory women, including subjects with PCOS [25] and those with altered signaling along the hypothalamic-pituitary-ovarian axis. First-line treatments for this group of patients include oral ovulation-inducing agents, which reduce the biosynthesis of estrogens and consequently decrease the negative hypothalamic feedback on Gonadotropin-Releasing Hormone (GnRH) and Follicle-Stimulating Hormone (FSH) [26]. Such drugs are either selective estrogen receptors modulators, such as clomiphene citrate (CC), or third-generation AI, such as Letrozole or Anastrozole. DCI may induce ovulation in PCOS patients in a similar way to Letrozole [27], and such an effect may be dose-dependent [28]. While low-dosage administration of DCI to PCOS patients exhibits negligible effects, doses as high as 1200 mg/day for 6–8 weeks successfully induced ovulation in up to 44% patients, compared to a control group taking placebo [29]. Such effect seems independent of BMI, as it was observed also with lean women [30]. Despite these pieces of evidence, the underlying mechanism of the ovulation induction after DCI treatment is still controversial. Besides the decreased activity of aromatase, also lowered levels of insulin may reduce estrogens production and release, restoring the physiological functioning of the hypothalamic-pituitary-ovarian axis [31]. Indeed, metformin is able to induce ovulation in PCOS patients as it reduces the insulin-stimulated production of androgens and the insulin-dependent activity of aromatase [32]. Decreased insulin levels also improve the efficacy of ovulation stimulation protocols either with gonadotropins [33] or AIs [34]. In rationalizing the effect of DCI on inducing ovulation, the contribution of both mechanisms should be considered, even though they occur with different timing.

4.2. Ovarian hyperstimulation syndrome (OHSS)

Although quite uncommon, OHSS represents one of the most serious complications of ovulation induction during in vitro fertilization (IVF) protocols. The syndrome is triggered by an exaggerated ovarian response to either exogenous (early-onset) or endogenous (late-onset) human Chorionic Gonadotropin (hCG), which induces the abnormal release of factors that increase vascular permeability [35]. In particular, luteal overexpression of Vascular-Endothelial Growth Factor (VEGF) seems to be directly associated with the onset of OHSS during stimulation protocols [36,37]. The consequent higher vascular permeability may lead to transudation of protein-rich fluid from the blood vessels mainly into the peritoneum. Pathological features range from mild symptoms, such as abdominal discomfort, to life-threatening complications in

the most severe cases. Treatments largely vary, depending on the severity of the clinical manifestations, and generally include cancellation of the stimulation cycle, with a profound economic and psychological burden for the patients [38]. Moreover, pregnancy-related complications are significantly higher in women who had suffered from OHSS, compared to IVF controls [39]. Prevention is therefore of paramount importance during fertilization programs and requires the assessment of risk factors and close monitoring of risk markers. Clinicians quite generally consider unusually elevated estrogen levels as both indicators for increased risk of OHSS and one possible etiological factor [40]. Strategies to reduce the occurrence of OHSS in subjects at risk include postponing (*coasting*) the hCG injection until the estradiol levels fall below a safe threshold [41]. Estrogen reduction with short-term Letrozole treatment (5 days) before hCG administration proved to significantly reduce the incidence of OHSS in high-risk women undergoing assisted reproduction [42]. Interestingly, analogous results were obtained with insulin sensitizers. Indeed, long-term treatment (>30 days) with metformin during ovarian stimulation also significantly reduced the risk of OHSS [43]. In this context, the potential use of DCI as a primary strategy, or more likely as an adjuvant treatment to the current management, may help to reduce the hyperestrogenism and the risk of OHSS.

4.3. Uterine leiomyomas (fibroids)

These are common benign tumors of the uterus and affect 20–30% of women in the reproductive age. Studies on leiomyoma cells found overexpression of the enzyme aromatase, compared with normal cells of the myometrium [44], with consequent high estrogen levels and increased expression of progesterone receptors [45]. Even though surgery represents the main curative treatment for large and symptomatic leiomyomas [46,47], the management of symptoms prevents stress and surgery-related complications. Reducing estrogen levels has been found particularly useful, as a hypoestrogenic environment proved to inhibit the proliferation of uterine myoma cells [48] and to reduce the size of uterine leiomyomas in women after the menopausal transition [49]. Treatment with aromatase inhibitors, such as Letrozole, was more effective than hormonal therapy in reducing fibroid volume and decreasing the duration and the volume of fluid absorbed during surgery [50]. On that basis, the use of DCI may reduce the pro-estrogenic microenvironment at level of the uterine myoma cells, and alleviate symptoms and signs by providing a potential effective adjuvant strategy to reduce fibroids' volume.

4.4. Endometriosis

The abnormal growth of endometrial-like tissue, glands, and stroma outside the uterine cavity is a frequent gynecological disorder that causes pelvic pain and may lead to infertility [51]. Endometriosis occurs primarily in women of reproductive age and tends to disappear after menopause when endogenous estrogen production decreases. However, recurrent endometriosis may be also detected in post-menopausal women. Researchers demonstrated that both ectopic and eutopic endometrial tissues in women with endometriosis express high levels of aromatase [52–54], causing the progression of this estrogen-dependent disease and the occurrence of a pro-inflammatory microenvironment [55]. Thus, the endometrium becomes a major intracrine source of estrogen, stimulating the progress of the disease [56]. Indeed, reducing systemic levels of estrogens halts the growth of endometriotic tissue, relieving the pain symptoms [57]. Recent findings suggest that drugs that inhibit the aromatase activity, such as Letrozole and Anastrozole, may have potential benefits in the treatment of endometriosis [58–60]. Indeed, they proved to significantly reduce both pelvic pain, lesion size, and endometrioma volume. However, AI treatments are more expensive and less tolerable than conventional therapies [61]. Similarly, insulin sensitizers seem to have beneficial effects on endometriosis status, comparable to AIs in the animal model: indeed, both *in vitro* and *in vivo* studies on rat endometriosis model demonstrated that treatment with metformin reduces the inflammatory response and the activation of aromatase, and stimulates the regression of endometriotic lesions [62,63]. In this scenario, by direct and indirect inhibition of aromatase, DCI treatment may play a beneficial role to reduce the pro-estrogenic local microenvironment typical of endometriosis and, most importantly, could be used in association with other drugs in order to reduce their doses and consequently the known potential side effects.

4.5. Breast and endometrial tumors

Both types of tumors have several risk factors in common. The majority of breast cancers and type-1 endometrial cancer express estrogen receptors and the risk of both is higher in women with elevated endogenous estrogen levels [64,65]. Moreover, both *in vitro* and *in vivo* investigations suggest that insulin and insulin-like growth factors (IGFs) may have a role in endometrial carcinogenesis, acting synergistically with estrogens [66]. Women with risk factors, especially those who had previous breast or endometrial cancer, represent a target for therapeutic intervention. By reducing estrogen levels, AIs proved to be effective, even more than Tamoxifen, in decreasing the long-term recurrence of estrogen-dependent cancer and the related mortality rate [67]. However, the benefits of treatments with AIs for extended periods must be carefully evaluated in light of the possible negative effects of hypoestrogenic status, especially on bone health. On that basis, DCI may have a potential application for a long-term modulation of aromatase activity after AIs termination, particularly in obese women with increased activity of aromatase even in the postmenopausal period [68].

4.6. Polycystic ovary syndrome

PCOS is generally characterized by insulin resistance, which entails a reduced MI-to-DCI conversion in most organs [69,70]. The ovaries represent an exception because they never develop low insulin sensitivity, resulting in ovarian hyperinsulinemia [71]. As a consequence, PCOS patients show an altered granulosa-to-theca layer ratio in the follicles, with the theca

layer being anomalously thicker than granulosa [72]. Hyperinsulinemia increases the LH signal on theca cells, boosting the biosynthesis of testosterone and leading to a hyperestrogenic state [73]. Moreover, hyperinsulinemia in PCOS women leads to increased MI-to-DCI conversion and to decreased MI/DCI ratios in theca cells [8] and follicular fluid [74]. In a recent review [69], Genazzani concluded that a tailored treatment with MI and DCI should be advisable to restore the altered inositol ratio in the ovaries of PCOS women. In particular, supplementation with MI and DCI, combined in the 40:1 physiological ratio [75–78], has been found to improve the phenotype and the fertility profile of such patients. However, administration of DCI to insulin-resistant PCOS patients may prove beneficial and reduce the systemic insulin levels only in the short term; conversely, longer treatment with DCI may lead to reduced expression of aromatase, with the potential increase of testosterone levels [16] and the consequent worsening of PCOS symptomatology. The available evidence on PCOS women suggests that the effect of DCI on aromatases may be a further explanation of the lower effectiveness of DCI alone supplementation in these patients.

4.7. Senile hypogonadism and male infertility

The pathological decreased levels of circulating testosterone in men can alter physical characteristics and the reproductive function. The condition leads to reduced libido, erectile dysfunction, infertility, lack of energy, reduced lean muscle mass with associated body fat gain, gynecomastia, impaired cognition, and psychological problems [79]. Hypogonadism may arise from testicular diseases (primary) or from the dysfunction of the hypothalamic-pituitary-gonadal axis (secondary). Hormonal imbalance in men over 40 years may bring to reduced production of gonadotropins, LH in particular, and to the onset of secondary hypogonadism (known as late-onset hypogonadism) [80]. AIs proved to decrease the negative feedback of estrogens on the hypothalamus and stimulate the release of endogenous gonadotropins. In particular, treatments with AIs seem to ameliorate the hormonal status and to reduce both mastalgia and gynecomastia [81], ameliorating the clinical condition of men with secondary hypogonadism. In this view, whether DCI's action as an inhibitor of aromatase should be confirmed, this compound could be evaluated as a novel potential treatment strategy for male hypogonadism and related subfertility, aiming to increase androgen levels, particularly in men with obesity-related secondary hypogonadism [82].

5. Safety

To the best of our knowledge, specific data concerning the safety of DCI are unavailable. The lack of studies that consider or specifically investigate the topic prevents to set boundaries to the therapeutic profile. Based on the aforementioned studies on DCI, we can confidently infer that the administration of DCI up to 1–2 g/day, even for extended periods of time, is safe and devoid of relevant side effects. However, the intake of DCI over this threshold may influence the metabolism of steroid hormones, altering the physiologic menstrual cycle. The activity on aromatase, in particular, may possibly cause an increase of androgen levels and the consequent worsening of the symptoms in women with PCOS.

6. Conclusions

Besides carrying the intracellular message of insulin, DCI influences the steroid biosynthesis by inducing the testosterone production and modulating the aromatase activity. Such an effect on aromatase may be exploited in the treatment and prevention of all the conditions that are generally treated with AIs and/or insulin sensitizers. The use of DCI prevents the buildup of a hypo-estrogenic environment, avoiding the onset of side effects generally associated with AI therapies.

7. Expert opinion

Accumulating evidence suggests that biologically active metabolites of DCI are deeply involved in modulating steroidogenesis, even though the exact underlying mechanisms remain to be elucidated. In particular, recent data proved that DCI, besides being one of the intracellular second messengers of insulin, affects the activity of aromatase and regulates the androgen-to-estrogen ratio. The mechanistic insights lately reported by Sacchi et al. demonstrated that DCI downregulates the gene expression of the enzyme [17], rather than inhibiting its activity. Hence, DCI modulates the levels of estrogens, and DCI treatment may reduce them without completely blocking their biosynthesis. In a recent publication, we speculated about a possible similar therapeutic role of DCI compared to AIs [18], even though the effects are markedly different and DCI administration avoids the onset of hypoestrogenic states. Because of such an advantage over AIs, investigating the potentialities of DCI treatments for estrogen-dependent conditions is particularly appealing. In our opinion, future studies should be planned to assess the potential therapeutic effect of DCI administration on the symptoms of gynecological benign conditions such as endometriosis and uterine fibroids, as well as the preventive activity on precancerous lesions. Likewise, DCI treatment should be evaluated as an adjuvant strategy to AIs in the post-operative management of estrogen-sensitive tumors, or as a prevention protocol for subjects presenting with risk factors. Specifically, obese women, particularly in menopause, may benefit from a double advantage of the long-term supplement of DCI due to the effects on insulin resistance and to the modulation of the activity of the aromatase in adipose tissue. Nevertheless, particularly in fertile age, we take the opportunity to underline that long-term treatments with elevated doses of DCI may cause an unwanted increase in testosterone levels as a secondary effect. Such occurrence should be considered in evaluating the therapeutic approach with DCI, especially for those pathological conditions whose symptoms may be exacerbated by increased androgen levels, such as PCOS [16]: in these cases, we believe that the mechanism of action fails to support a rationale for the use of high doses of DCI alone as successful treatment.

DCI is an insulin-sensitizing agent, and short-term supplementation may be beneficial to treat hyper-androgenic or

hyper-estrogenic states associated with hyper-insulinemia. Nevertheless, the effect of DCI on aromatase can further explain why short-term treatments with DCI successfully induced ovulation in a dose-dependent manner in PCOS patients [28]. DCI is able to reduce the negative feedback on gonadotropin release not only by reducing systemic insulin levels but even reducing estrogen production. On that basis, we suggest that a similar effect may potentially be observed also in non-PCOS, non-IR anovulatory women, which may benefit from an initially increased FSH release by reduced estrogens. Thus, evaluating the possibility of high-doses supplementation with DCI and the duration of the treatment would be an interesting option in such patients.

Interestingly, hypogonadal men can benefit from an increase of testosterone, which is unwanted in women. They, indeed, exhibit low testosterone levels and altered androgen-to-estrogen ratios, suffering from reduced fertility and altered physical characteristics. Treatment with DCI, by reducing the androgen-to-estrogen conversion, may help to decrease the negative feedback of estradiol on the hypothalamus also in this case, stimulating the release of endogenous gonadotropins and improving the clinical condition of men with secondary hypogonadism. Particularly in men with obesity-related hypogonadism, the long-term supplement of DCI may be useful for both to the effects on insulin resistance and on the activity of the aromatase in adipose tissue [82–84].

Based on the available evidence, we suggest that the administration of DCI should be carefully considered in light of the patient's specific situation (the type of condition, insulin status, gender), evaluating the proper dosage and treatment duration accordingly. Further studies based on DCI administration should aim to define the safety profile and the therapeutic rationale for each specific condition, such as estrogen-dependent gynecological pathologies (endometriosis, uterine fibroids) as well as high estrogen production in obese male and female patients, a possible target of DCI supplementation. In this scenario, potential hypo-estrogenic state caused by high levels of DCI may play a positive role to counteract the typical pro-estrogenic microenvironment associated with the progression of endometriosis and enlargement of fibroids. Nevertheless, targeted trials will be mandatory in order to define the best dose/duration of DCI therapy for each disease, considering the patients' condition.

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