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Could myo-inositol soft gel capsules outperform clomiphene in inducing ovulation? Results of a pilot study

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Abstract. – OBJECTIVE: Insulin resistance is known to worsen polycystic ovarian syndrome (PCOS). The management of insulin resistance is crucial in the treatment of PCOS and insulin-sensitizing molecule as myo-inositol (MYO) seems to have promising effects. The aim of our pilot study was to study whether supplementation with MYO can improve patients' sensitivity to clomiphene citrate (CC) in terms of ovulation and pregnancy rates.

PATIENTS AND METHODS: This study included 26 patients with PCOS, eligible to ovulation induction with CC. All of them received MYO in combination with CC and folic acid, following the usual protocol. Results concerning ovulation and pregnancy rates were compared to those from our historical cohort of PCOS patients treated with CC alone.

RESULTS: Ovulation rate was significantly higher with MYO+CC than with CC alone (65.5% *vs.* 42%, *p*=0.0001). The number of patients sensitive to 50 mg/d was 54% with MYO *vs.* 40% in our reference cohort (NS). The total resistance rate was 19% *vs.* 27% in the reference cohort (NS). Cumulative pregnancy rate with MYO+CC was 53.8% *vs.* 42.2% with CC alone (NS). Pregnancy rates per initiated cycle were 16.1% with MYO *vs.* 12.6% in the historical cohort (NS).

DISCUSSION: Although the differences were not significant for most outcomes, probably due to the small number of patients, our pilot study seemed to show a benefit of supplementation with MYO during ovulation induction with CC in PCOS patients.

CONCLUSIONS: This study proves the great interest of a RCT and re-opens the possibilities of insulin-sensitizing agents in the treatment of anovulatory patients with PCOS, such as natural products like MYO.

Key Words
Inositol, Clomiphene citrate, PCOS, Anovulation, Infertility, Insulin resistance.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of ovulation disorder, hyperandrogenism, and infertility since it affects approximately 5 to 15% of women of childbearing age[1]. Metabolic syndrome with insulin resistance is found in 60 to 70% of women with PCOS. Insulin resistance is now admitted to be an aggravating factor of PCOS through a mechanism of compensatory hyperinsulinemia, which stimulates steroidogenesis and results in amplification of hyperandrogenism and abnormal folliculogenesis[2-4].

The management of insulin resistance is essential in the treatment of PCOS. It is based primarily on lifestyle dietary rules and insulin-sensitizing agents such as Metformin, which is indicated in France only in case of glucose intolerance and type 2 diabetes. Many studies in other countries have shown that the combination of metformin with lifestyle and dietary rules enabled weight loss, decreased subcutaneous fat tissue, and even allowed to regain regular menstrual cycles[5]. According to the meta-analysis of Tang et al[6], Metformin seems to improve clinical pregnancy rates but not live birth rates when used alone, compared to a placebo or when combined with Clomiphene Citrate (CC) versus CC alone. Therefore, the role of Metformin to improve reproductive outcomes in women with PCOS seems to be limited. Moreover, Metformin's side-effects (mainly gastro-intestinal) usually limit the patients' compliance to the treatment.

However, other molecules seem to be interesting in the management of insulin resistance. Among these molecules, myo-inositol (MYO) appears to have promising effects in PCOS[7,8]. Inositol is a polyalcohol from the group of vitamins B,

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with 9 different stereoisomers whose MYO. This molecule presents *in vivo* an insulin-like action by acting as an intracellular mediator of insulin by means of inositolphosphoglycans[9]. In PCOS, there would be a deficit in MYO at the ovarian level, exacerbating the symptoms[10].

According to the consensus of Thessaloniki[11], CC is the first line treatment of dysovulation in PCOS but about 25% patients do not respond with the highest dose of CC. The purpose of this pilot study was to study whether supplementation with MYO improves the patients' sensitivity to CC or not, in terms of ovulation and pregnancy rates. If the benefit of MYO were suggested, this would support the realization of a randomized controlled trial

Patients and Methods

This is a preliminary prospective non-comparative study.

Population

Patients were included at the time of the infertility evaluation, conducted in our hospital. Inclusion criteria were: -1) age between 18 and 40, -2) seeking pregnancy, -3) PCOS defined by the Rotterdam criteria: antral follicle account (CFA) >19 per ovary and/or a cycle disorder and/or clinical hyperandrogenism (at least two of the three criteria)[12], -4) eligibility to CC, meaning that the infertility was only due to PCOS, the rest of the female and male investigations were normal. Exclusion criteria were: BMI > 35 kg/m², another cause of oligo-anovulation, history of ovarian drilling after the initial assessment, non-compliance to supplementation by MYO.

Hormonal and Ultrasound Work up

Hormonal measurements and a vaginal ultrasound were performed between cycle days 2-5. The following parameters were collected at baseline: -1) clinical parameters: age, rank and duration of infertility, smoking, waist circumference, weight, height and BMI; -2) biological parameters: serum prolactin, LH, FSH, estradiol (E2), 17-hydroxyprogesterone (17OHP), dehydroepiandrosterone-sulfate (DHEA-S), androstenedione (A) and total Testosterone (TT) levels were measured by immunoassays as described previously[13]. Serum Anti-Müllerian Hormone (AMH) levels were assessed using the second-generation enzyme immunoassay AMH-EIA (ref A16507) provided by Beckman Coulter Immunotech (Villepinte, France), as described previously[14]; -3) Ultrasound (U/S) parameters: antral follicle count (AFC) and ovarian volume (OV). For every patient, U/S examination was performed with a Voluson E8 Expert (General Electric Systems, VELIZY, France) with a 5-9 MHz transvaginal transducer. U/S measurements were done at the time of the U/S, according to as standardized protocol. After determination of the longest medial axis of the ovary, the length and thickness were measured, and the OV was calculated as described previously[15]. For each ovary, the total number of all visible follicles smaller than 10 mm in diameter (AFC) was counted by a slow and continuous scanning of the ovary, from one border to the other, in longitudinal cross-section.

Treatment Protocol

Patients were informed (oral and written information) at baseline, about the composition of the soft capsule (600 mg of myo-inositol + 100 microg of folic acid), the dosage (twice a day), the potential effect of MYO, and the need to start the treatment before stimulation. The consent was collected by oral (no written consent was necessary since MYO is not considered as a drug but as a dietary supplement). MYO supplementation consisted in 2 capsules a day, each capsule containing 600 mg of MYO + 100 microg of folic acid daily, given in addition to the 400 microg/day of folic acid usually prescribed (i.e., a total of 600 microg/day of folic acid). Treatment was started on average 2 months before the initiation of CC and was continued throughout the treatment with CC. Treatment with CC followed the usual protocol: initial dose of 1 tablet per day (50 mg/d) started on Day 2 (D2) of the cycle and continued up to D6. Follicle size(s) and number were checked by ultrasound at D12 and the occurrence of an ovulation or not was asserted by a serum progesterone (P) assay between D22 and D24. In the absence of ovulation (i.e., P<4 ng/ml), CC dose was increased to 2 and if necessary to 3 tablets/day. Compliance was monitored at each attempt.

Outcomes

Results obtained with CC+MYO were compared to those from our historical cohort, recently re-evaluated retrospectively (unpublished results). This cohort included 109 patients, with the following characteristics: median age: 28 years, median BMI: 23.7 kg/m², and median waist cir-

Table I. Population features.

	Median	5th-95th percentiles
Age	26	22.3- 31.8
BMI (kg/m^2)	25.5	18.1-34.6
WC (cm)	87	64-102.1
SHBG (nmol/l)	37.1	14.6-68.4
HDL-C (g/l)	0.5	0.37-0.7
LH (IU/l)	7.2	3-12.5
FSH (IU/l)	4.8	3.4-6.1
LH/FSH ratio	1.6	0.7-2.6
Estradiol (pg/ml)	41	25-63.8
AMH (pmol/l)	107	46.3-175.4
Testosterone (ng/ml)	0.42	0.12-0.63
Androstenedione (ng/ml)	2.17	0.87-2.73
DHAS (µmol/l)	4.2	2.31-8.94
17OHP (ng/ml)	0.63	0.35-0.99
AFC	32.7	15.3-54.4
Ovarian area (cm^2)	6.7	0.6-8.4

cumference: 79 cm. The number of cycles studied was 366.

Thus were compared: the ovulation rate per cycle, the percentage of patients sensitive to 1, 2 or 3 tablets of CC, the percentage of patients totally resistant to CC (i.e., no ovulation under 150 mg/d) and the pregnancy rate per cycle (defined by a positive βhCG test and confirmed by ultrasound at 6 weeks of gestation). A cycle was considered ovulatory when P level at day 22-24 was greater than 4 ng/mL. CC sensitivity was defined by ovulation on at least two consecutive cycles. CC resistance was defined by the absence of ovulation with the maximum dose of 3 tablets/d.

Statistical Analysis

The Chi 2 test was used to compare the results to the historical cohort. The MF-Calc software (*http://www.spc.univ-lyon1.fr/mfcalc/*) was used to determine the size of the population that would allow sufficient statistical power for a further randomized controlled study.

Results

26 patients had at least 1 CC cycle. The characteristics of these patients were summarized in Table I. 87 cycles were studied. The results were compared with those obtained in our historical cohort of 109 patients and 366 cycles.

The ovulation rate, regardless the dose of CC, was 65.5% (57/87) *versus* 42% (154/366) in our historical cohort (p=0.0001).

The number of patients who ovulated at least two consecutive times under CC+MYO was 20/26 (77%) *vs.* 80/109 (74%) in the reference cohort (NS). The rate of sensitivity and resistance to CC are shown in Figure 1. The number of patients sensitive to 50 mg/d was 14/26 (54%) *vs.* 44/109 (40%) in our reference cohort (NS). The total resistance rate to CC was 5/26 (19%) *vs.* 29/109 (27%) in the reference cohort (NS). The cumulative pregnancy rate with CC+MYO was 53.8% (14/26) *vs.* 42.2% (46/109) (NS). Pregnancy rate per initiated cycle was 16.1% (14/87) *vs.* 12.6% (46/366), respectively (NS). Pregnancy rate per ovulatory cycle was 24.6% (14/57) *vs* 29.9% (46/154) in the reference cohort (NS).

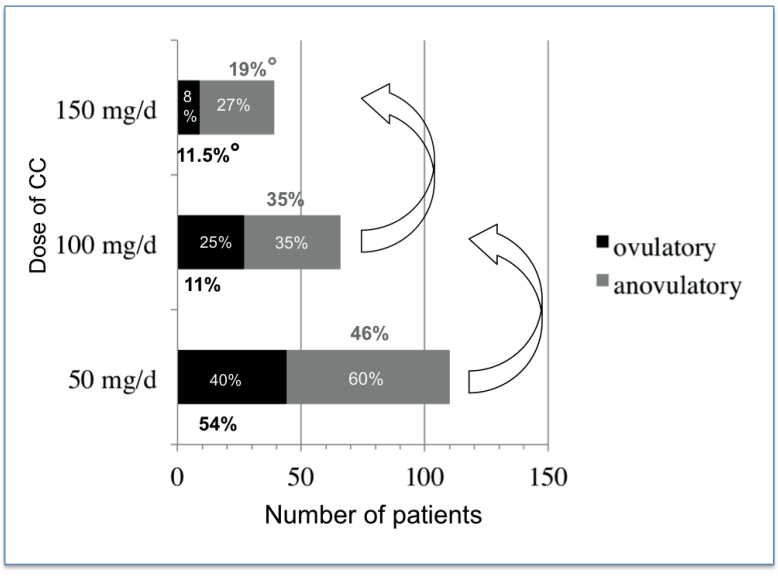

Figure 1. Numbers of patients sensitive *(in black)* or not *(in grey)* to CC alone in our reference cohort. Percentages are shown inside the bars. Percentages of patients sensitive *(in black)* or not *(in grey)* to CC+MYO are shown outside the bars. No significant difference was found between the reference cohort and the group of patients treated with CC+MYO, at any dosage.
°: the total (30.5%) is not 35% because one patient switched to *rec*FSH instead of CC 150 mg/d.

No patient discontinued supplementation by MYO. Only one patient had to stop CC because of visual disturbances at 150 mg/d.

Discussion

Although the differences were not significant for most outcomes, probably due to the small sample of patients, our pilot study seems to show a benefit of supplementation with MYO during ovulation induction with CC in PCOS patients.

Indeed, 77% of our patients ovulated with CC+MYO, which is slightly higher than in our baseline cohort which exhibits comparable results with the literature[16-22]. The total resistance to CC in our population was 19%, which is less than the 27% of our baseline cohort and similar results of the literature: 27%, 22.5%, 35 % and 25% in the series of Homburg et al[20], Imani et al[17], Ghobadi et al[23] and Eijkemans et al[24], respectively. The sensitivity to 50 mg day of CC seemed better with CC+MYO than with CC alone since the ovulation rate was 54% *vs.* 40% in our historical cohort. For Rostami-Hodjegan et al[25] this rate was 46%. The cumulative pregnancy rate per patient was higher (53.8%) than in the reference cohort (42.2%) and in the literature[18,20,26,27].

Pregnancy rate per ovulatory cycle was slightly lower (24.6%) than in our historical cohort (29.9%) but comparable to those in the literature: 22% and 24% in the series of Hammond et al[26]and Lopéz et al[28], respectively. Pregnancy rate per cycle was higher (16.1%) than in our reference cohort (12.6%) and in the study of Homburg et al (14.6%), who compared CC versus gonadotropins[29].

Our study was designed to evaluate the feasibility of a larger trial to study whether adding MYO to CC lowers or not the resistance to CC. Indeed, no study has focused so far on the interest of MYO during ovulation induction with CC. Two previous studies[30,31] focused on the effect of MYO in combination with *rec*FSH on ovulation induction outcomes and another one compared MYO alone *vs.* MYO + CC[32].

Although the number of cycles was rather small (n = 87), MYO seemed to improve sensitivity to CC in patients with anovulation due to PCOS, when compared to our large reference group (366 cycles). It should be noticed that this is not a comparative study. Our preliminary results allowed us to calculate the required number of patients for a randomized controlled trial (RCT) in order to have sufficient power. To detect a significant increase of 50% in the ovulation rate with CC+MYO *vs.* CC alone, each arm should include 85 patients (for a power of 80% and an α risk of 5%, tailed test). To detect a significant increase of 30% in ovulation rate with 50 mg of CC+MYO *vs* 50 mg CC alone, each arm should include 267 patients (for a power of 80% and an α risk of 5%, tailed test). To detect a significant decrease of 30% in the resistance to CC with CC+MYO *vs.* CC alone, each arm should include 431 patients (for a power of 80% and an α risk of 5%, tailed test). To detect an increase of 50% in the pregnancy rate per cycle with CC+MYO *vs.* CC alone, each arm should include 552 patients (for a power of 80% and an α risk of 5%, tailed test).

It is important to note that no data from the literature reports metabolic or hormonal effect of folic acid among women of childbearing age. Therefore it seems unlikely that supplementation with 0.6 mg of folic acid (instead of 0.4 mg usually) could be responsible for the results observed with MYO. Last, the adherence of the patients was almost complete. Only two patients refused to be included in our protocol.

Conclusions

This study proves the feasibility of a RCT and the need to focus particularly on the contribution of MYO during ovulation induction with CC. The different data from the literature and the promising results of this study re-open the possibilities of insulin-sensitizing agents in the treatment of anovulatory patients with PCOS, including the use of natural products like MYO.

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Conflict of interest

The authors declare no conflict of interest.

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