Gynecological Endocrinology, April 2010; 26(4): 275–280

informa
healthcare

PCOS

Insulin sensitiser agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women

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(Received 30 April 2009; accepted 23 September 2009)

Abstract

Objective. The aim of this study was to compare the effectiveness of myo-inositol (MYO) and metformin, in monotherapy or in association with recombinant follicle stimulating hormone (r-FSH), in the treatment of menstrual irregularities, chronic anovulation, and female infertility in patients with polycystic ovary syndrome (PCOS).

Materials and methods. One hundred twenty patients were randomly treated with metformin 1500 mg/day orally ($n = 60$), or 4 g MYO plus 400 μg folic acid daily ($n = 60$), continuously. If no pregnancy occurred, r-FSH (37.5 units/day) was added to the treatment for a maximum of three attempts.

Results. Fifty percent of the patients who assumed metformin restored spontaneous ovulation, 18.3% of these obtained pregnancy. The remaining 42 patients were treated with metformin plus r-FSH. Pregnancy occurred in a total of 11 women (26.1%). The total pregnancy rate was 36.6%. Sixty-five percent of the patients treated with MYO plus folic acid restored spontaneous ovulation activity, 30% of these obtained pregnancy. The remaining 38 patients were treated with MYO, folic acid plus r-FSH. Pregnancy occurred in a total of 11 women (28.9%). The total pregnancy rate was 48.4%.

Conclusions. Both metformin and MYO, can be considered as first line treatment for restoring normal menstrual cycles in most patients with PCOS, even if MYO treatment seems to be more effective than metformin.

Keywords: *Metformin, myo-inositol, ovulation induction*

Introduction

Polycystic ovary syndrome (PCOS) is a medical condition affecting approximately 6–10% of women in fertile age [1].

It is characterised by chronic anovulation, hyperandrogenism and typical polycystic ovaries at ultrasound evaluation [2,3].

Chronic anovulation usually manifests as menstrual irregularities such as oligo/amenorrhea. The most evident consequence of chronic anovulation is female infertility [4].

Excess androgens cause dermatological alterations such as acne, hirsutism, seborrhea and androgenic alopecia [5].

In the long term, PCOS is associated with an increased risk of developing hypertension, dyslipidemia, impaired glucose tolerance or type 2 diabetes [6,7] and may also confer an increased risk of cardiovascular disease [8–10].

The pathogenesis of PCOS is poorly understood. No single etiologic factor may be fully accounted for the spectrum of abnormalities in PCOS. Many recent studies have focussed on the impaired glucose tolerance that affects 30–40% of patients with PCOS. A great body of evidence has demonstrated the important role of reduced insulin sensitivity in many patients with PCOS. Interestingly, both obese and lean women with PCOS manifest insulin resistance independent of fat mass [11].

Insulin sensitising compounds, such as metformin, pioglitazone and troglitazone have been proposed as putative treatments for hyperinsulinemia-induced dysfunction of ovarian response to endogenous gonadotropins to improve ovulation, menstrual cycles and hyperandrogenemia [6,12,13].

An inositol phosphoglycan molecule containing D-chiro-inositol (DCI) is known to have a role in activating enzymes that control glucose metabolism [14].

Indeed, a defect in tissue availability or altered metabolism of DCI or inositol phosphoglycan mediators have been found in women affected by PCOS and may contribute to their insulin resistance [15,16].

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ISSN 0951-3590 print/ISSN 1473-0766 online © 2010 Informa UK Ltd.
DOI: 10.3109/09513590903366996

Myo-inositol (MYO) is an isoform of inositol and belongs to the vitamin B complex.

Elevated concentrations of MYO in human follicular fluid appear to play a role in follicular maturity and provide a marker of good-quality oocytes [17].

The outcome of the use of insulin-sensitising agents in monotherapy or in association with clomiphene citrate for ovulation induction is still debated in term of increasing rate of both spontaneous ovulation and pregnancy [18–23].

Previous studies have demonstrated that MYO is capable of restoring spontaneous ovarian activity, and consequently fertility, in most patients with PCOS [24,25].

During a follow-up period of 6 months, ovulation rate and consequently pregnancy were not found significantly different in patients with PCOS treated as first line with MYO or Clomiphene [26].

Our aim is to compare the effectiveness of two different insulin-sensitiser agents, inositol and metformin, in the treatment of menstrual irregularities, chronic anovulation and female infertility during a treatment period of 6 months.

Furthermore in the women who failed to conceive, we evaluated the effects of continuing the treatment with insulin-sensitiser agents in association with very low-dose recombinant follicle stimulating hormone (r-FSH), for inducing mono-ovulation.

Materials and methods

A total of 120 women, aged <35 years, with PCOS, defined by Rotterdam Criteria, were enrolled in the study from June 2006 and June 2008.

All patients attended our IVF Department for infertility that lasted for a period of more than 14–16 months.

Exclusion criteria were as follows:

1. Other medical condition causing ovulatory dysfunction: hyperprolactinemia or hypothyroidism, or androgen excess, adrenal hyperplasia or Cushing's syndrome, were excluded by hormonal tests.
2. Tubal defects: in fact all women underwent assessment of tubal patency.
3. Semen parameters defects: all male partners were evaluated with two different sperm semen samples, without finding any defect.

Anovulation was ascertained by weekly plasma progesterone concentration below 2.5 ng/ml. Thus, at the end of diagnostic procedures, it was determined that the most likely cause of the couple subfertility was only ovulation dysfunction.

All patients gave a written informed consent to the procedure, and the study was approved by the Local Ethics Committee.

MYO was approved as experimental treatment in consideration of the effects that belongs to B-vitamin group, and in the literature there are plenty of records for its use in patients with PCOS and its safety in reproduction [20,27].

Women with PCOS were randomly treated with metformin 1500 mg/day (Glucophage®, Merck Pharma), orally, or 4 g, MYO plus 400 μg folic acid (Inofolic®, Loli Pharma, Rome, Italy) as soluble powder, daily, continuously, until the end of the study or a positive pregnancy test. Patients were instructed to register their menstrual bleeding throughout the follow-up period of 6 months and were instructed about optimal sexual intercourse scheme to achieve a pregnancy. Primary endpoint was to evaluate the restoration of spontaneous ovarian activity, by weekly serum progesterone dosage, as well as transvaginal ultrasound scan documenting presence of follicular growth or luteal cyst.

Progesterone levels higher than 8.0 ng/ml were considered significant for spontaneous ovulation.

Secondary endpoints were myo- or metformin-resistance (percentage of patient who did not restore spontaneous ovulation), pregnancy rate (documented by positive β-hCG plasma level and foetal heart beat on ultrasound scan) and abortion rate.

If no pregnancy occurred, patients continuing insulin-sensitiser treatment underwent ovulation induction with recombinant FSH (Gonal-F®, Merk-Serono, SUI) for a maximum of three attempts. A very low-dose protocol (37.5 U/day) beginning from the day two of menstrual flow in a step up regime was selected.

Urinary HCG (5000 UI Gonasi® AMSA, Rome, Italy) was administrated until no more than two follicles of a diameter >17 mm were detected on ultrasound.

Statistical analysis

Randomisation was performed with 'intention to treat' criteria. Baseline characteristics were evaluated with t-test. Ovulation rate was compared with a Wilcoxon matched-pairs signed-ranks test. Pregnancy rate was evaluated using χ^2-test. $p < 0.05$ was considered statistically significant.

Results

There were no significant differences in baseline variables among the study groups (Table I). Seven of 60 subjects (10.9%) in the metformin group dropped out of the study because of the development of side effects and loss of follow up, and 4 of 60 subjects (8.3%) dropped out of the MYO group because of loss of follow up. (p: n.s.).

Sixty patients were treated with daily doses of 1500 mg of metformin for 6 months.

Fifty percent (30 of 60) of these patients restored spontaneous ovulation activity documented by follicular growth at ultrasound evaluations and increased serum progesterone concentrations in the luteal phase. In the patients with restored monthly menstruation, ovulation occurred after a mean 16.7 ($\pm$2.5) days from the first day of the menstrual cycle.

Pregnancy occurred spontaneously in 11 (18.3%) of these patients. Seven women dropped out. The remaining 42 patients were treated with 1500 mg of metformin plus recombinant FSH for a maximum of three cycles. Pregnancy occurred in a total of 11 women (26.1%). Nine of these pregnancies occurred in the metformin resistant patients ($n = 23$), whereas two in the group which had ovulation restored with metformin alone (Figure 1).

Table I. Clinical, hormonal and metabolic data of anovulatory women with PCOS.

	Metformin	Myo-inositol	*p*
Age (years)	29.7 $\pm$ 6	29.1 $\pm$ 5.6	0.67
BMI	24.9 $\pm$ 2.7	25 $\pm$ 2.1	0.84
WHR	0.90 $\pm$ 0.4	0.88 $\pm$ 0.3	0.62
Duration of Infertility	20.1 $\pm$ 3.5	22.2 $\pm$ 2.5	0.25
FSH (mIU/ml)	7.5 $\pm$ 1.8	7.2 $\pm$ 2.0	0.65
LH (mIU/ml)	9.1 $\pm$ 2.1	9.6 $\pm$ 2.5	0.1
TSH (μU/ml)	2.9 $\pm$ 0.3	2.6 $\pm$ 0.8	0.35
PRL (ng/ml)	8.7 $\pm$ 2.5	9.2 $\pm$ 2.3	0.15
E_2 (pg/ml)	38.0 $\pm$ 9.5	34.5 $\pm$ 8.2	0.15
P (ng/ml)	0.8 $\pm$ 0.6	0.5 $\pm$ 0.3	0.32
17-OHP (μg/l)	1.8 $\pm$ 0.35	2.0 $\pm$ 0.7	0.28
T (ng/ml)	0.9 $\pm$ 0.5	1.1 $\pm$ 0.7	0.11
A (ng/ml)	1.4 $\pm$ 0.4	1.8 $\pm$ 0.4	0.10
DHEAS (ng/ml)	2.721 $\pm$ 435	2.456 $\pm$ 480	0.55
SHBG (nmol/l)	27.0 $\pm$ 6.4	27.2 $\pm$ 5.6	0.75
Fasting glucose (mg/dl)	77.5 $\pm$ 10.5	80.1 $\pm$ 8.9	0.53
Fasting insulin (μU/ml)	20.3 $\pm$ 4.5	21.2 $\pm$ 4.8	0.40

Metformin assumption was interrupted in case of positive pregnancy test.

The total pregnancy rate was 36.6% (22 women of 60). Five of the 22 pregnancies (22.7%) evolved in spontaneous abortion at 9 weeks of gestation.

The 60 patients treated with 4 g/day of MYO plus 400 μg/day of folic acid for 6 months. Sixty-five percent of these patients restored spontaneous ovulation activity documented by follicular growth at ultrasound evaluations and increased serum progesterone concentrations in the luteal phase. Ovulation occurred after a mean of 14.8 ($\pm$1.8) days from the first day of the menstrual cycle. Pregnancy occurred spontaneously in 18 (30%) of these patients. Four women dropped out. The remaining 38 patients were treated with 4 g/day of MYO, 400 μg/day of folic acid plus recombinant FSH in small doses (37.5 U/day) from the second day of menstrual flow for three cycles. Pregnancy occurred in a total of 11 women (28.9%). Eight of these pregnancies occurred in the MYO resistant patients ($n = 17$), whereas three in the group which had ovulation restored with MYO alone (Figure 2).

The total pregnancy rate was 48.4% (29 women of 60).

Six of the 29 pregnancies (20.6%) evolved in spontaneous abortion.

The efficacy in restoring regular ovulation was evaluated comparing both the percentage of patients who responded the treatment and the median length of follicular phase in metformin ant in MYO group: ovulation occurred after a mean 16.7 ($\pm$2.5) days from the first day of the menstrual cycle in the metformin group and after a mean 14.8 ($\pm$1.8) in

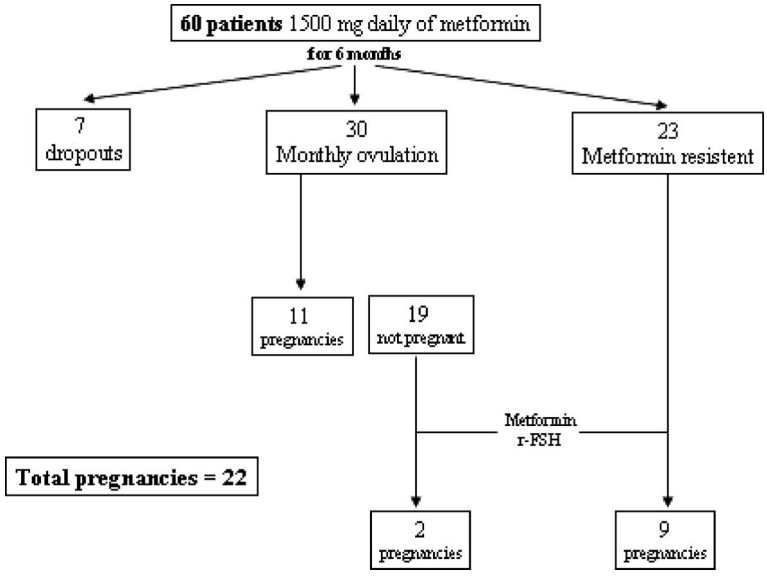

Figure 1. Flow chart describing the number of pregnancies in the group of patients receiving metformin and metformin +r-FSH.

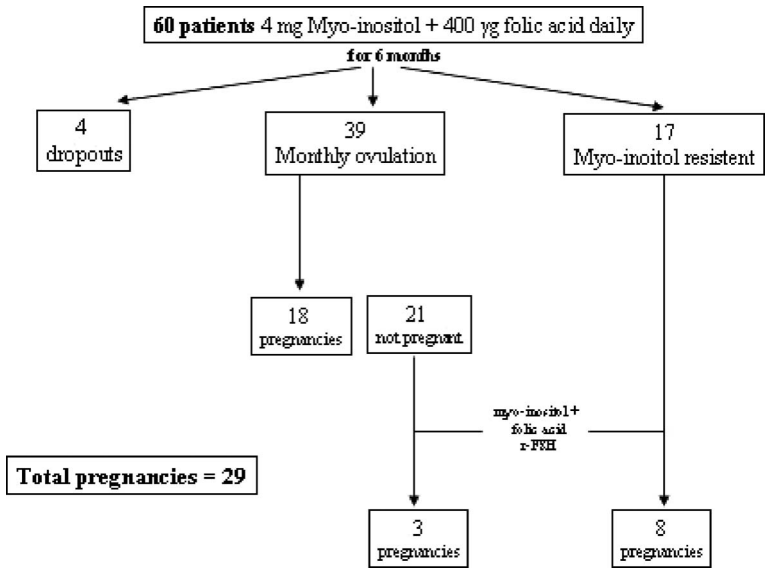

Figure 2. Flow chart describing the number of pregnancies in the group of patients receiving MYO and folic acid and MYO and folic acid +r-FSH.

Table II. Out come of insulin sensitiser treatment in monotherapy.

	MYO ($n=60$)	MET ($n=60$)	p
Patients with restored monthly ovulation	39	30	0.09
Median length of follicular phase	14.8 ± 1.8	16.7 ± 2.5	0.003
Patients with failure to ISC treatment	17	23	0.24
No. of pregnancy (%)	18/60 (30%)	11/60 (18.3%)	0.13
No. of pregnancy/ patients with restored ovulation	18/39 (46.1%)	11/30 (36.6%)	0.42

ISC, insulin sensitiser compounds.

Table III. Outcome of insulin sensitiser plus r-FSH treatment.

	MYO ($n=38$)	MET ($n=42$)	p
No. of pregnancy (%)	11/38 (28.9%)	11/42 (26.1%)	0.72
No. of pregnancy/ patients with restored ovulation with ISC	3/21 (14.2%)	2/19 (10.5%)	0.71
No. of pregnancy/ patients previous failed ovulation with ISC	8/17 (47.1%)	9/23 (39.1%)	0.61

Table IV. Overall pregnancy rate.

	MYO	MET	p
Pregnancy rate	29/60 (48.3%)	22/60 (36.6%)	0.19
Abortion rate	6/29 (20.6%)	5/22 (22.7%)	0.86

the MYO group. The median between metformin and MYO differs significantly ($p < 0.003$) from zero with a Wilcoxon matched-pairs signed-ranks test.

Furthermore, we compare outcome of the treatment evaluating the pregnancy rate, both alone or in association with r-FSH. The results are reported in the Tables II–IV.

Pregnancy rate of insulin sensitiser in monotherapy did not differ in the two groups (p: n.s. with χ^2 test, Table II).

Comparing patients who failed to conceive with metformin or MYO in monotherapy, no significant difference in pregnancy was noted when recombinant FSH was added to induce mono-ovulatory cycles (Table III). In the patients who failed to conceive, statistical analysis was performed also for two subgroups: the patients who previously responded or not with spontaneous ovulation to monotherapy. No significant difference were found among these groups (data not reported). The overall pregnancy rate and abortion rate did not differ in the two groups (Table IV).

Discussion

PCOS is one of the most common endocrine disorders affecting women of reproductive age [1]. The syndrome was considered to be mainly a reproductive disorder, but important data have highlighted its metabolic implications [2]. The association between insulin resistance, compensatory hyperinsulinemia and hyperandrogenism has provided insight into the pathogenesis of PCOS [4]. The cellular and molecular mechanisms of insulin resistance in PCOS have been extensively investigated, and it is evident that the major defect is a decrease in insulin sensitivity secondary to a post-binding abnormality in insulin receptor-mediated signal transduction, with a less substantial, but significant, decrease in insulin responsiveness [28–30]. Many interventional studies have demonstrated a positive

effect of insulin-sensitising agents such as metformin and troglitazone in the treatment of PCOS. Recently, attention has been given to inositolphosphoglycan (IPG) mediators as post-receptor mediators or second messengers of insulin signalling. It is demonstrated that DCI administration increases the action of insulin in patients with PCOS, thereby improving ovulatory function and decreasing serum testosterone concentration [20]. MYO, that is a precursor of DCI, is also effective on PCOS similarly to DCI and has positive effects on hyperinsulinemic, PCOS women in reproductive age, both on spontaneous ovulation and metabolic parameters [13,24–26,31].

This study was designed to test and compare the effects of metformin and MYO on restoring spontaneous ovulation and menstrual cycles and increasing rate pregnancy.

Our data support the hypothesis of a primary role of both metformin and MYO as first-line therapies for restoring a spontaneous ovulation in women with PCOS.

These antidiabetic drugs, increasing glucose utilisation in insulin-sensitive tissues, are equally useful in the reduction of both insulin resistance and circulating androgens as well as restoring ovulation.

Trials of both metformin or MYO in monotherapy, followed by very low-dose r-FSH ovulation induction in case pregnancy is not achieved in a few months, are a reasonable approach, to reduce risk of ovarian hyperstimulation syndrome and multiple pregnancy [32].

Moreover, a comparative analysis between the two groups showed an overall higher rate of pregnancies (48.3% vs. 36.6%) in the group treated with MYO, even if not statistically significant. This findings enforce the hypothesis of a primary role of IPG as second messenger of insulin signal and demonstrate that MYO oral supplementation, similarly to DCI administration, induces the reduction of insulin levels probably acting on the higher availability of such precursors of IPG and thus, ameliorating the performances of this second messenger of insulin signal. Results are supported by clinical studies. Inositol is correlated to oocyte maturity through Ca^{2+} signalling [17].

The present results are in line with other studies evaluating insulin-lowering medications, and in particularly different isoforms of inositol, suggesting the positive effect that these compounds play on spontaneous ovarian activity, increasing also pregnancy rate. Furthermore, we found that MYO seems more effective than metformin suggesting that an endocellular defect of the precursor of inositolphosphoglycan (IPG) such as MYO and/or DCI might trigger the compensatory hyperinsulinemia in most, though not all, PCOS subjects.

In conclusion, insulin sentitiser agents, both metformin and MYO, can be considered first-line treatment in most patients with PCOS, for restoring normal mentrual cycles [33]. Co-administration of those compound are useful in improving the monoovulation rate in women treated with r-FSH.

Furthermore, oral administration of MYO is a simple and safe treatment that seems positively correlated also to oocyte matutity, with the possibility in addition to increase spontaneous fertility.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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