Maitake Mushroom (Grifola frondosa) Extract Induces Ovulation in Patients with Polycystic Ovary Syndrome: A Possible Monotherapy and a Combination Therapy After Failure with First-Line Clomiphene Citrate

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Abstract

Background: Insulin resistance is a prominent feature of polycystic ovary syndrome (PCOS), and insulin-sensitizing drugs are used to induce ovulation. Recently, it was reported that an extract from Maitake mushroom (Grifola frondosa) improves insulin resistance.

Objectives: The objective was to explore the effects of Maitake extract (SX-fraction: MSX) to induce ovulation in patients with PCOS in comparison with and in combination with clomiphene citrate (CC).

Design: We conducted an open trial with 80 patients with PCOS at three clinics in Japan. Seventy-two (72) new patients were randomly assigned to receive MSX or CC monotherapy for up to 12 weeks. Eighteen (18) patients who did not respond to MSX or CC were subjected to combination therapy of MSX and CC for up to 16 weeks. Eight (8) patients with documented history of failure to CC received combination therapy from the beginning. Ovulation was assessed by ultrasonography.

Results: Twenty-six (26) patients in the MSX group and 31 in the CC group were evaluated for ovulation. The ovulation rates for MSX and CC were as follows: 76.9% (20/26) and 93.5% (29/31), respectively by the patients (NS), and 41.7% (30/72) and 69.9% (58/83), respectively, by the cycles (p = 0.0006). In the combination therapy, 7 of 7 patients who failed in MSX monotherapy and 6 of 8 patients who failed in CC monotherapy showed ovulation.

Conclusions: The present study suggests that MSX alone may induce ovulation in PCOS patients and may be useful as an adjunct therapy for patients who failed first-line CC treatment.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in approximately 7%–8% of reproductive women, which has been characterized clinically by menstrual dysfunction, hyperandrogenism, and metabolic complications.1

Criteria for diagnosis of PCOS remain controversial. Recent consensus in 2003 by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine has broadened the diagnostic criteria incorporating the ultrasound findings (the Rotterdam Criteria). It requires two of the three following features: oligomenorrhea or amenorrhea, presence of polycystic ovarian morphology on ultrasound, and clinical or laboratory evidence of hyperandrogenism.2 The diagnosis of PCOS in the absence of hyperandrogenism has generated controversy3: Women with oligomenorrhea and polycystic ovarian morphology but without hyperandrogenism do not meet the criteria, making it difficult to apply these criteria to Asian women with less incidence of hyperandrogenism due to different genetic backgrounds.4 Under these circumstances, in 2007 the Japan Society of Obstetrics and Gynecology proposed criteria for Japanese women with PCOS, who

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presented with menstrual abnormality due to the presence of polycystic ovarian morphology on ultrasound, and laboratory evidence of hyperandrogenism and/or elevated basal luteinizing hormone (LH) and normal basal follicle-stimulating hormone (FSH). The serum LH and FSH levels are required to be >7 mIU/mL and LH/FSH >1, with an exception that obese women with the BMI >25 can be diagnosed with PCOS if the LH/FSH ratio is greater than 1.5

Clomiphene citrate (CC) has been regarded for many years as the first-line standard medication to induce ovulation in patients with PCOS. A prospective follow-up of thin women with ovulatory dysfunction has shown high conception rates approaching 50% in ovulatory responders treated with CC after three cycles of treatment, and 75% within nine cycles.6 Successful ovulation is achieved in 70%–80% of women, and 40%–50% will conceive.7

Insulin resistance and compensatory hyperinsulinemia are prominent features of PCOS. Increased insulin concentration leads to hyperandrogenism because of increased production of ovarian androgen and decreased synthesis of sex hormone binding globulin. Metformin, a drug for hyperinsulinemia and insulin resistance, has been used to induce ovulation in women with PCOS.8,9 A meta-analysis of 13 trials with metformin suggests that it is an effective treatment for anovulatory women with PCOS.10

Maitake mushroom (Grifola frondosa) has been known to have a potent immune stimulatory effect, and an active extract called “D-fraction” has been identified.11 Also, Maitake mushroom has been reported to reduce elevated blood glucose levels, reduce blood pressure, and modulate serum lipids,12–19 and a novel bioactive extract called “SX-fraction” (MSX), a water-soluble glycoprotein with an average molecular weight of 20,000 Da, was identified as an extract to improve insulin resistance recently.20–22

In this study, we investigated MSX for its effects to induce ovulation as a monotherapy in comparison with CC, and as a combination therapy with CC after failure of either MSX or CC monotherapy in women with PCOS.

Materials and Methods

Materials

MSX tablets, containing 18 mg of MSX and 250 mg of dried Maitake mushroom powder, was a generous gift of Maitake Products, Inc. (East Rutherford, NJ). Subjects were instructed to take 3 tablets, 3 times a day orally between meals during the trial period.

Patients and protocol

Eighty (80) subjects, who were diagnosed with PCOS during April 2006 through October 2007 at one of our 3 clinics, J.T. Chen Clinic, Loma Linda Clinic, or Sophia Ladies Clinic, and diagnosed with PCOS, were enrolled in this study. The inclusion criteria were the following: (1) 18–35 years of age; (2) diagnosed with PCOS, indicated by the presence of oligomenorrhea, and hyperandrogenism and/or elevated basal LH and normal basal FSH, polycystic ovaries revealed by ultrasonography; (3) no diabetes mellitus; and (4) no hypercholesterolemia. This study was approved by the Institutional Review Board at J.T. Chen Clinic, and informed consent was obtained from all participants prior to the study.

Subjects were then divided randomly into two groups: 36 women were assigned to receive MSX and another 36 to received CC. The study was open label as it was difficult to make a placebo because of the characteristic appearance and odor of MSX. Six (6) subjects—4 women in MSX and 2 women in CC groups—were excluded from safety and ovulation analyses because they did not return to the clinics after the entry visit. Another 12 subjects—6 women in MSX, 3 women in CC, and 3 women in concomitant treatment—were excluded from ovulation analysis due to dropout (4 subjects) or deviation from the protocol (8 subjects).

At the time of entry, all the subjects were tested in the equivalent of the follicular phase of the menstrual cycle around 10 days after the onset of last menstruation. Baseline laboratory testing of the subjects was performed as described below, and their weights, heights, and blood pressures while supine were measured as well. The presence of polycystic ovarian morphology and more than 12 follicles with 2–9 mm in diameter, was also assessed on ultrasound.23 In subjects without recent menses, withdrawal bleeding was induced with a course of oral medroxyprogesterone acetate before the initiation of study medication.

Subjects were administered MSX on the first day of menses continued for up to 12 weeks or 3 cycles, or CC at a daily dose of 50 mg from days 5 to 9 of menses, and repeated up to 3 cycles. Subjects then returned for a visit every 2 weeks to have ultrasonography taken for follicular and endometrial response. Ovulation was also assessed by ultrasonography.

Subjects who did not respond to either MSX or CC were further subjected to combination therapy. The total of 15 subjects, including 7 from the 36 MSX group and 8 from the 36 CC group, have undergone combination therapy for up to 16 weeks or 4 cycles.

Biochemical assay in blood

Baseline laboratory testing was performed on the subjects who had fasted overnight. All specimens were analyzed in the Mitsubishi BCL Laboratory (Tokyo, Japan), while FSH, LH, prolactin (PRL), and testosterone were measured by radioimmunoassay.

Data management and statistical analysis

Data entry was conducted at each clinical site and double-checked at the Department of Gynecology and Obstetrics at Odaira Memorial Tokyo Hitachi Hospital. Data analysis was performed at Anzai & Associates, and either Wilcoxon rank-sum test or Fisher’s exact test was used for statistical analysis.

Results

Background of subjects and ovulation effects by treatment

Twenty-six (26) subjects in the MSX group and 31 in the CC group were eligible for ovulation analysis in this study. Between the 2 groups, there were no statistical differences in the baseline background valuables (Table 1).

Ovulation was observed in 20 of 26 subjects treated with MSX (the ovulation rate was 76.9%) and 29 of 31 subjects with CC (93.5%), although such a difference was not statistically
significant (p = 0.124). When the ovulation rate was compared by the cycles, we found 30 ovulated cycles in 72 cycles (41.7%) in the MSX group, whereas there were 58 ovulated cycles in 83 cycles (69.9%) in the CC group (Table 2). The difference between the 2 groups was then statistically significant (p = 0.0006).

**Combination effects on ovulation with MSX and CC**

Fifteen (15) subjects who failed to respond to monotherapy with either MSX (7 cases) or CC (8 cases) have undergone combination therapy using MSX combined with CC. In this therapy, we found that 7 of 7 cases that failed in MSX treatment and 6 of 8 that failed in CC treatment resulted in ovulation (Table 3).

**Conception and adverse events**

Three (3) of 26 subjects in the MSX group and 8 of 31 subjects in the CC group wished to get pregnant. A total of 3 subjects conceived during the treatment; 2 of the 3 subjects conceived by MSX only, and the rest by combination treatment after CC monotherapy failed.

As to adverse events of MSX treatment, we observed only 2 subjects with slight epigastralgia, which disappeared during the course of continued treatment under careful observation.

**Discussion**

This study demonstrated that MSX was capable of inducing ovulation in patients with PCOS. The ovulation rate of 76.9% in the MSX group was very impressive even compared to that in the CC (93.5%) (despite p = 0.124). However, when the ovulation rate was compared by the cycles, 41.7% ovulated cycles in the MSX group was significantly lower than 69.9% in the CC (p = 0.0006).

It has been shown that Japanese women with PCOS exhibited less obesity and hirsutism compared with those in United States and Italy,24 who exhibit a disproportionately high LH secretion with relatively constant low FSH secretion. This suggests a generalized dysregulation of ovarian androgen secretion augmented by insulin; in fact, the increased insulin concentration is known to lead to hyperandrogenism because of the increased production of ovarian androgen but decreased synthesis of sex hormone binding globulin.

Metformin, a drug for hyperinsulinemia and insulin resistance, has been thus used to induce ovulation in women with PCOS.8,9 A meta-analysis of 13 trials with metformin indicates that it is an effective treatment for anovulation in women with PCOS.10 Ovulation was indeed achieved in 46% of women with metformin, similar to the benefit conferred by CC10 (compared with only 24% in the placebo group).

MSX, a water-soluble glycoprotein with an average molecular weight of 20,000 Da, is reported to modulate blood glucose levels, reduce blood pressure, normalize serum lipids, and enhance insulin sensitivity in spontaneously hypertensive rats by intraperitoneal glucose tolerance and insulin challenge tests.20–22 The response of circulating glucose and insulin concentrations was examined at different time periods during an intraperitoneal glucose tolerance test. Ingestion of MSX produced lower circulating levels of glucose after the glucose challenge without increase of circulating insulin levels. Compared to control, significantly lower circulating glucose levels were seen in rats consuming pioglitazone and MSX after the insulin challenge whether or not glucose was given concomitantly.22 Insulin sensitizers, including thiazolidinediones,25 rosiglitazone,26 pioglitazone,27 metformin,28 and d-chiro-inositol,29 have also been shown to increase ovulation and reduce hyperandrogenism in women.

Table 3. Ovulation Effect by Combination Treatment

<table>
<thead>
<tr>
<th>First-line treatment</th>
<th>MSX</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>No. of ovulated subjects</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>(%)</td>
<td>100.0</td>
<td>75.0</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>No. of ovulated cycles</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>(%)</td>
<td>82.6</td>
<td>55.6</td>
</tr>
</tbody>
</table>

MSX, Maitake SX-fraction; CC, clomiphene citrate.

Table 1. Background Data of Subjects

<table>
<thead>
<tr>
<th></th>
<th>MSX Mean value ± SD</th>
<th>CC Mean value ± SD</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.0 ± 3.8</td>
<td>26.0 ± 4.4</td>
<td>23 NS</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>18</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.8 ± 4.9</td>
<td>159.0 ± 5.5</td>
<td>31 NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.8 ± 6.9</td>
<td>52.8 ± 8.9</td>
<td>31 NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.6 ± 3.0</td>
<td>20.9 ± 3.3</td>
<td>31 NS</td>
</tr>
<tr>
<td>&lt;25</td>
<td>24</td>
<td>58</td>
<td>27</td>
</tr>
<tr>
<td>≥25</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>99.8 ± 10.8</td>
<td>100.8 ± 8.6</td>
<td>31 NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>61.7 ± 10.0</td>
<td>62.3 ± 8.3</td>
<td>31 NS</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>66.7 ± 7.4</td>
<td>70.6 ± 7.7</td>
<td>31 NS</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>84.2 ± 5.6</td>
<td>85.0 ± 6.4</td>
<td>30 NS</td>
</tr>
<tr>
<td>IRI (µU/mL)</td>
<td>6.1 ± 4.8</td>
<td>7.7 ± 8.9</td>
<td>30 NS</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.30 ± 1.04</td>
<td>1.67 ± 2.01</td>
<td>30 NS</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>57.3 ± 20.5</td>
<td>54.5 ± 19.6</td>
<td>30 NS</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.0 ± 1.1</td>
<td>5.6 ± 1.1</td>
<td>30 NS</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>17.8 ± 6.3</td>
<td>14.1 ± 4.7</td>
<td>30 NS</td>
</tr>
<tr>
<td>Testosterone (pg/mL)</td>
<td>70.6 ± 28.5</td>
<td>62.7 ± 33.6</td>
<td>30 NS</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>11.8 ± 5.8</td>
<td>10.3 ± 4.7</td>
<td>30 NS</td>
</tr>
</tbody>
</table>

*Statistical difference was assessed by Wilcoxon rank-sum test.

Table 2. Ovulation Effect by Treatment

<table>
<thead>
<tr>
<th></th>
<th>MSX</th>
<th>CC</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>26</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>No. of ovulated subjects</td>
<td>20</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>76.9</td>
<td>93.5</td>
<td>0.124</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>72</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>No. of ovulated cycles</td>
<td>30</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>41.7</td>
<td>69.9</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

*Statistical difference was assessed by Fisher’s exact test.

MSX, Maitake SX-fraction; CC, clomiphene citrate.
with PCOS. The ovulation rate of 41.7% by MSX (Table 2) was no less than those of insulin sensitizers, indicating that MSX could be an alternative treatment in women with PCOS.

A commercial product was used in this study as we have done a doctor initiative clinical trial. The constituents of the product were Maitake extract containing MSX and Maitake powder. The Maitake powder was used as an excipient to avoid using artificial excipients; however, adding Maitake powder may affect the MSX insulin-sensitizing effect by other minor unidentified ingredients. The manufacturing of the product takes place at a Pharmaceutical GMP (Good Manufacturing Practice) certified manufacturer where appropriate quality control and quality assurance is performed. The Maitake extract used in this study was obtained according to a proprietary process of the manufacturer (U.S. Patent 7,214,778). Every lot of Maitake extract is analyzed by high-performance liquid chromatography, and the content of MSX is standardized to be more than 18%. It is not certain whether the result obtained with this particular extract is also applicable to other Maitake extracts on the market whose manufacturing processes are either not disclosed or different from that of our Maitake extract.

In this study, who patients failed in monotherapy of either MSX or CC demonstrated a significant improvement in ovulation with the combination therapy of these two agents (Table 3). CC has been the “gold standard” first-line treatment to induce ovulation in women with PCOS for many decades, showing a high degree of efficacy in up to 80%; however, some women with PCOS were still found to be resistant to this agent, leading to no ovulation. It is thus uncertain whether insulin sensitizer, either alone or in combination with CC, would improve the ovulation rate in PCOS. Similarly, the actual efficacy of metformin alone or its combination with CC on induction of ovulation in PCOS patients has been shown to be somewhat inconsistent and inconclusive.

It is rather interesting that our combination therapy of MSX and CC resulted in the high, improved ovulation rate of 87% (13/15) in those patients who had previously failed with either MSX or CC monotherapy. This appears to be promising and may have clinical implications of MSX in a better, improved therapeutic modality for those women with PCOS. Moreover, it is important to explore whether MSX exhibits a synergistic effect with CC in a similar mechanism with other insulin sensitizers besides CC. Such a study is certainly warranted.

We did not intend to examine the effect on infertility in this study. In fact, only 11 subjects—3 of 26 subjects in the MSX group and 8 of 31 in the CC group—wished to be pregnant. Nevertheless, 3 of 11 such infertile subjects conceived during the treatment: 2 of 3 subjects by MSX only, and the rest by combination treatment after failure with first-line CC. A multicenter trial that directly compared the effects of CC and metformin for infertility as a single agent found CC to be more effective than metformin overall. The trial showed that the live birth rate in CC was higher than metformin and no additional benefit of combination treatment on live birth rate was seen compared with CC alone. We have observed the 3 of 3 subjects who conceived after treatment with MSX in this study; hence, further study on infertility by MSX alone or in combination with CC after failure with first-line CC treatment was warranted. Our study showed that the adverse events of MSX treatment were mild and well tolerated.

**Conclusions**

The present study supports the clinical utility of MSX as a monotherapy for induction of ovulation in women with PCOS. Additionally, its combination therapy with CC may provide a beneficial effect, further facilitating ovulation in those who initially failed with monotherapy with either MSX or CC. Thus, MSX may have significant clinical implications in women with PCOS.

**Disclosure Statement**

Hideo Anzai serves as a senior manager for Maitake Products, Inc. None of the other authors declare a personal or financial conflict of interest.

**References**


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