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= Original Article =

Ovulatory effects of an extract from Maitake mushroom in patients with polycystic ovary syndrome

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ABSTRACT

This study aimed to determine the effects of an extract from Maitake mushroom ("SX-fraction", SX) on ovulation in patients with polycystic ovary syndrome (PCOS). Thirty patients were randomly assigned to either of the following two treatment arms for three months: the SX group (n = 15), which prescribed 207 mg/day of SX, and the control group (n = 15), which received 7.5 g/day of Shakuyaku-Kanzo-To (SKT; a Chinese herbal medicine). Ovulation was judged by the change of basal body temperature and ultrasonography. During the treatment period, 66.7% of the patients in the SX group ovulated compared with 30.8 % in the SKT group; however, the difference was not statistically significant (p = 0.0581). During the same period, ovulation was confirmed in 22 out of 45 menstrual cycles (48.9%) in the SX group, while for the SKT group ovulation was confirmed in 15.4% (P = 0.0011). Estradiol and the estradiol/testosterone ratio also showed a statistically significant increase (P = 0.032 and 0.038, respectively) when those compared before and after treatment with SX. The SX group was 14 times more likely than the SKT group to induce ovulation (95% CI: 1.093 - 196.45). These results summarized; 1) SX was effective in the treatment of PCOS and 2) The SX regimen improved the ovulation rate independent of insulin resistance. In conclusion, SX appears to promote aromatization of testosterone to estradiol in granulosa cells.

Key words: polycystic ovary syndrome (PCOS), treatment, Maitake mushroom extract (SX-fraction), insulin resistance, aromatization

INTRODUCTION

Polycystic ovary syndrome (PCOS), characterized clinically by oligomenorrhea or anovulation, hyperandrogen, and polycystic ovaries, is one of the most common endocrinopathies and a major cause of infertility, affecting approximately 4.6% (3.5%-11.2%) of women of reproductive age [12]. Although there is an internationally accepted definition of PCOS [19], ethnic differences in the disease should be considered. Therefore, in 2007, the Japan Society of Obstetrics and Gynecology proposed slightly different criteria for Japanese women [10].

In the treatment of PCOS, clomiphene citrate (CC) has been used for many years as the standard medication to induce ovulation [17]. According to the step-by-step clinical protocol, if CC treatment fails (unsuccessful ovulation or pregnancy), patients are recommended for consecutive gonadotropins, ovarian surgery, and in vitro fertilization. Such treatments, however, are expensive and associated with some risk of side effects.

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On the other hand, it has been found that hyperinsulinemia might contribute to hyperandrogenism, representing metabolic and hormonal aspects of PCOS. Therefore, drugs to improve hyperinsulinemia and insulin resistance (e.g., metformin) have been introduced for the treatment of PCOS [2, 26]. These drugs, however, have been reported to be associated with several adverse effects, as in the case of metformin, which is widely known to induce lactic acidosis, a rare side effect [3, 4]. It is then desirable to develop or explore certain medications with less adverse reactions.

Maitake is an edible mushroom belonging to the family Polyporaceae in the order Aphyllophorales. Maitake has been shown to be capable of lowering blood glucose levels [9, 13–15, 18, 21] and an extract responsible for that activity has been identified and named "SX-fraction". It is a glycoprotein with an average molecular weight of around 20,000 Daltons, and has been reported to improve insulin resistance and associated clinical symptoms, including PCOS. In the present study, we examined SX-fraction, an extract from Maitake (*Grifola frondosa*) mushroom, as a potential alternative therapeutic modality for PCOS. SX-fraction proved to be effective in ovulation induction in our pilot study, which was presented at the 22nd annual meeting of Traditional Medicines in Tokyo, 2005 [24].

MATERIALS AND METHODS

Materials

In the present study, we used the following plant source products: 1) SX-fraction (Maitake Products, Inc. NJ, U.S.A.) and 2) Shakuyaku-Kanzo-To (SKT), a commonly prescribed Japanese traditional herbal medicine (Tsumura & Co., Tokyo, JAPAN), with less adverse reaction, as used for the treatment of PCOS [20].

Study design and subjects

An unblinded prospective randomized controlled 3-month trial was designed and conducted at Loma Linda Clinic from March to December 2007. This study included 30 Japanese women (21-37 years old) with newly diagnosed PCOS. Diagnosis of PCOS was based on the 2007 Japan Society of Obstetrics and Gynecology criteria, as follows: 1) menstrual abnormality, 2) polycystic ovary and 3) hyperandrogenism and/or elevated basal luteinizing hormone (LH) and normal basal follicle stimulating hormone (FSH). As for LH and FSH, both LH $\geq$ 7 mIU/ml and LH/FSH $\geq$ 1 are required; in the case of obesity (body mass index, BMI $\geq$ 25), however, LH/FSH $\geq$ 1 is acceptable to diagnose PCOS [10]. Trans-vaginal ultrasound was performed by the same examiner to diagnose PCOS morphologically, i.e., 1) the number of follicles is more than 10, and 2) the sizes of follicles are less than 9 mm.

The patients who met the medical criteria were randomly allocated to either of the two treatment arms. Allocation was made by opening sealed envelopes that contained random assignment cards to either the SX-fraction group or the SKT group. The investigators and the patients were not blinded to the treatment arms, due to a difficulty in preparing indistinguishable placebos for SX-fraction and SKT. The patients were instructed to take SX or SKT three times before meals per day.

The basal body temperature (BBT) records of the subjects were examined and anovulations lasting for more than three months were confirmed. Patients with known history of hypogonadotropic hypogonadism, diabetes, or liver, renal, or heart disease were excluded from the study. Anovulatory patients were given medroxyprogesterone acetate (MPA) to induce a withdrawal bleeding, and a blood test was run to detect hormonal and metabolic disorders as a routine procedure. The respective heights, weights and BMIs of all patients were also recorded at entry into the study.

The study was approved by the Institutional Review Board at Inui Institute for Frontier Reproductive Medicine and Infertility, and informed consent was obtained from all participants prior to the study.

Medications and monitoring

The patients were assigned to two groups: the SX-fraction group (n = 15), which received 207 mg/day of SX-fraction, and the SKT group (n = 15), which received 7.5 g/day of SKT. The status of the patients' menstruation and ovulation was monitored by recording menstrual bleeding and BBT every morning. BBT has been confirmed as a relatively accurate method for retrospective identification of ovulation [16]. In addition, trans-vaginal ultrasonography was used to observe follicle development (a follicle size of $\geq$ 12 mm was considered a developing follicle) or ovulation. The participants were instructed to maintain any routine exercises and/or follow the dietary regimen they have been on before joining this study. During the study two patients from the SKT group dropped out upon personal

Table 1. Characteristics of 28 women with the polycystic ovary syndrome before and after treatment with SX-fraction and SKT

	SX-fraction (n=15)			SKT (control) (n=13)		
	At baseline	After treatment	P	At baseline	After treatment	P
Age	31.53 ± 5.11	–	–	28.1 ± 4.42	–	–
BMI	23.01 ± 7.04	–	–	22.6 ± 5.18	–	–
LH	13.61 ± 4.36	11.41 ± 5.19	0.079	12.90 ± 4.87	11.90 ± 5.22	0.525
LH/FSH	2.43 ± 0.66	2.34 ± 0.96	0.614	2.51 ± 1.68	2.28 ± 1.03	0.619
T	56.95 ± 27.64	54.55 ± 30.59	0.517	35.71 ± 7.90	36.14 ± 10.79	0.917
E_2	39.72 ± 12.03	83.38 ± 71.37	0.032	37.17 ± 11.38	51.24 ± 34.95	0.209
PRL	10.95 ± 5.84	13.38 ± 4.35	0.252	10.31 ± 5.45	12.47 ± 4.61	0.124
E_2/T	0.87 ± 0.50	1.83 ± 1.62	0.038	1.09 ± 0.36	1.80 ± 1.78	0.186
FBS	82.47 ± 8.26	82.53 ± 7.10	0.970	82.42 ± 6.68	82.08 ± 6.84	0.852
IRI	5.61 ± 4.26	6.36 ± 4.73	0.193	7.18 ± 3.41	6.57 ± 6.03	0.548
HOMA-IR	1.21 ± 0.99	1.30 ± 0.98	0.603	1.47 ± 0.72	1.40 ± 1.40	0.781

Mean ± SD are presented.
SX-fraction; extract from Maitake mushroom, SKT; Shakuyaku-Kanzo-To, BMI; body mass index, T; testosterone, E_2; estradiol, PRL; prolactin, FBS; fasting blood sugar, IRI; fasting insulin, HOMA-IR: homeostasis model assessment for insulin resistance.

Table 2. Prognostic factors for ovulation

	Odds ratio	95% CI
Age	1.00	0.798–1.255
Treatment	14.66	1.093–196.452
LH	0.85	0.642–1.136
T	0.97	0.931–1.018
BMI $\geq$ 25	0.73	0.036–15.108
HOMA-IR $\geq$ 2	5.83	0.251–135.641

Treatment; SX-fraction (compared to SKT), T; testosterone,
CI: confidence Interval, HOMA-IR: homeostatic model assessment index,
BMI:body: mass index.

requests unrelated to a safety concern.

Assays

Blood was drawn in the morning, between day 3 and day 5 of the withdrawal bleeding or menstruation periods, and the samples were sent to the same laboratory for biochemical analysis. IRI (fasting insulin), FBS (fasting blood glucose), HOMA-IR (homeostatic model assessment index), E_2 (estradiol), LH, FSH, LH/FSH (LH to FSH ratio), T (testosterone), E_2/T (estradiol to testosterone ratio), and PRL (prolactin) levels were measured at baseline and after three months. A quantitative determination of the serum hormones was performed by chemiliuminescense immunoassay (CLIA).

Statistical Analyses

The continuous variables are expressed as means ± SD (standard deviation). Fisher's exact test was used to analyze the statistical difference between the treatment arms in "ovulation rate" (percentage of ovulated patients) and "ovulation cycle rate" (percentage of menstrual cycles with ovulation). Paired t-test (two-tailed) was used to assess the statistical difference between the values at baseline and those after the treatment in hormonal and metabolic parameters. Multiple logistic regression analysis was used to examine the effects of treatments after adjusting the effects of other factors. Statistical significance was accepted when p-value is less than 0.05. Any data on the two discontinued patients were excluded from the analysis. Data analysis was then performed using Statistics Package for Social Sciences (SPSS) Version 11.5 for Windows (IBM Japan, Tokyo, Japan).

RESULTS

Ten of the 15 patients in the SX-fraction group ovulated (ovulation rate = 66.7 %) during the treatment period compared with 4 of 13 patients in the SKT group (ovulation rate = 30.8 %), but the difference was not statistically significant (P = 0.0581) (Figure 1). On the other hand, ovulation was confirmed in 22 out of a total 45 cycles (ovulation cycle rate = 48.9%) in the SX-fraction group during that period. This rate was significantly higher (P = 0.0011) than that of the SKT group, which was only 6 out of a total 39 cycles (ovulation cycle rate = 15.4%) (Figure 2). These results show that SX-fraction was more effective than SKT in

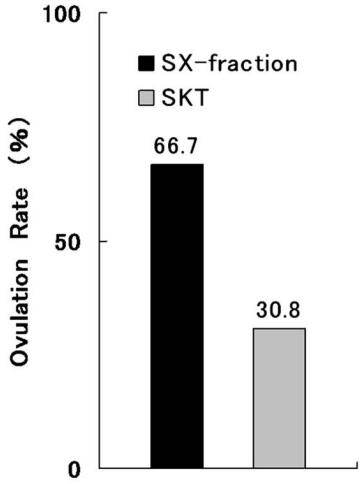

Figure 1. Ovulation rates in extract from Maitake mushroom (SX fraction) and Shakuyaku-Kanzo-To (SKT) groups
Data were calculated based on the number of patients with polycystic ovary syndrome who ovulated during the administrateion of SX fraction and SKT. The rates were not different significantly (P = 0.0581).

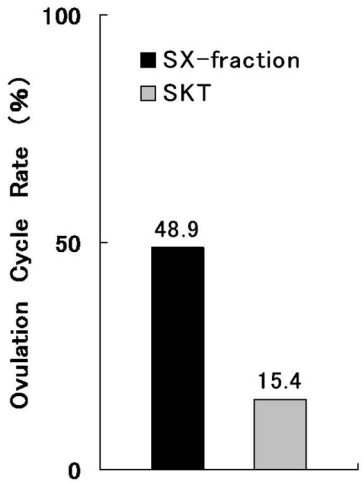

Figure 2. Ovulation cycle rates in extract from Maitake mushroom (SX fraction) and Shakuyaku-Kanzo-To (SKT) groups
Ovulation was confirmed 48.9% of total cycle in SX fraction and 15.4% in SKT. The rates were different significantly (P = 0.0011).

inducing ovulation in patients with PCOS.

Table 1 shows various parameters of the subjects (n = 28) before and after the treatment with SX-fraction and SKT (control). There were no statistically significant differences at baseline between the study groups. In the treatment group with SX-fraction, LH decreased from 13.61 ± 4.36 to 11.41 ± 5.19, although it was not statistically significant (P = 0.079). E_2 and the E_2/T ratio (a higher E_2/T ratio represents a greater aromatization activity) were significantly increased after administration of SX-fraction (P = 0.032 and 0.038, respectively). The results of multiple logistic regression analysis (Table 2) show that SX-fraction was almost 14 times more likely than SKT to induce ovulation (odds ratio of 14.66 at 95% confidence interval for 1.093–196.45).

DISCUSSION

Maitake is well known to have potent immune stimulatory and anti-tumor activities, which have been shown to be superior to those in other mushrooms. Moreover, such super- bioactivities of Maitake are known to stem from an active ingredient called "D-fraction" [8]. Besides this fraction, another bioactive compound has been recently isolated from Maitake and been so called "SX-fraction". It is a water-soluble glycoprotein with an average molecular weight of 20,000 Daltons. Recent reports have shown that SX-fraction is capable of modulating blood glucose levels [9, 13–15, 18, 21]. Other studies on SX-fraction have indicated that it might help reduce blood pressure [1, 11, 22, 23] and serum lipids [6, 11]. Thus, it is conceivable that SX-fraction may be a useful natural product to prevent the development of insulin resistance syndrome or metabolic syndrome. Furthermore, it may be used as an alternative agent for the treatment of PCOS.

Based on the findings of the current reproductive endocrinology, insulin resistance and excessive production of androgen from ovary are more likely to impede ovulation. Probably they could be the major contributors to the pathogenesis of PCOS. For example, insulin sensitizers such as metformin have been shown to improve the hormonal imbalances associated with PCOS, resulting in an improvement of ovulatory function. Nonetheless, some patients may experience gastrointestinal symptoms during metformin therapy, and in rare cases, metformin may cause fatal lactic acidosis. Accordingly, we investigated the ovulatory and endocrinological, metabolic effects of SX-fraction as a possible safer alternative treatment for PCOS. The results of multiple logistic regression analysis, adjusting the effects of confounding variables at baseline that might have affected the treatment outcomes, showed a remarkable treatment effect of SX-fraction (Table 2).

As far as hormonal and metabolic changes in the patients are concerned, E_2 and the E_2/T ratio were

significantly increased after administration of SX-fraction (Table 1). These results indicate that SX-fraction may increase the aromatization in the granulosa cells, which promotes the biosynthesis of E_2 from T, thereby resulting in the increased serum E_2/T ratio. Although SX-fraction has been reported to likely improve insulin resistance, no significant changes were observed in FBS, IRI, or HOMA-IR by SX-fraction in our PCOS patients. This discrepancy could be due, at least in part, to the limited sample size of patients with insulin resistance or other factors. The complete picture of the pharmacological effects of SX-fraction on PCOS remains to be elucidated; however, to a certain extent, SX-fraction may yet share some similarities with SKT. Takahashi and Kitao have reported a significant increase of the E_2/T ratio in PCOS patients who received SKT. This implies that SKT may increase the activity of aromatase, promoting the synthesis of E_2 from T and subsequently lowering the serum testosterone levels [20]. Fulghesu et al. reported that increased aromatization activity stimulated by exogenous gonadotropins is a reflection of hyperinsulinemic status in patients with PCOS [7]. We assumed that this may partially explain the pharmacological effects of SX or SKT on PCOS.

In addition, locally produced androgens and estrogens are important in the hormonal regulation of follicular development: increased E_2 and decreased T productions would facilitate the increase in follicle size. The decrease in E_2 production observed during atresia of the dominant follicle is believed to be the direct result of decreased aromatase activity in granulosa cells [25]. Our findings also suggest that SX-fraction appears to stimulate the conversion of T to E_2 (aromatization) and/or to reverse the inhibited aromatization of testosterone in granulosa cells, which demonstrates an antagonistic effect on follicular development.

Furthermore, it is known that race and ethnicity would influence the phenotypic manifestation of PCOS; for instance, Japanese women have been shown to express less clinical and biochemical hyperandrogenism. Therefore, it is of interest to investigate the effects of SX-fraction on PCOS with respect to racial and ethnical differences in the future.

Regarding the safety of SX-fraction, it would be reasonable to assume that the risk would be minimal for the following reasons. Firstly, SX-fraction is derived from an edible mushroom with a history of human experience for more than a thousand years. Secondly, SX-fraction showed no toxicity in single-dose and repeated-dose toxicity studies (unpublished in-house data; Maitake Products, Inc. NJ, U.S.A). Moreover, there have been no reports suggesting its possible toxicity in the laboratory and clinical studies with SX-fraction conducted so far [18, 21–23], and no serious adverse events have been reported since it was launched in the United States in 2004 (unpublished in-house data; Maitake Products, Inc. NJ, U.S.A.). SX-fraction was actually well tolerated without any problem in our present study.

A few limitations in the present study should be noted. Since the sample size of this trial was relatively small, it may reflect the wide range of the confidence interval of odds ratio in the multiple logistic regression analysis. Additionally, due to the small sample size of insulin-resistant subjects, i.e., 5 of 28 (17.8 %) of HOMA -IR$\geqq$ 2.0, we could not show a statistically significant effect of SX-fraction on the improvement of insulin resistance. However, the endocrinological mechanism of SX-fraction would be explained by increased aromatization after treatment. Further studies to explore the relationship between E_2, T, E_2/T, and insulin resistance are expected. Then, a large-scale study should be required to overcome such a limitation in the study (More recently, Chen et al. conducted a large scale study and confirmed the effect of SX-fraction to induce ovulation in PCOS patient) [5]. Furthermore, this study was not blinded, so that it is nearly impossible to rule out the involvement of some inevitable bias associated with investigators and/or patients.

In conclusion, SX-fraction was effective in anovulatory patients with PCOS, demonstrating the significant increases in E_2 and the E_2/T ratio. Multiple logistic regression analysis also indicated that SX-fraction regimen indeed improved the ovulation rate after adjusting for the effect of other factors.

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