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Effects of Unkei-to on FSH, LH and Estradiol in Anovulatory Young Women with Hyper- or Hypo-Functioning Conditions

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Abstract: Eight weeks treatment with Unkei-to induced a significant increase in plasma follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol levels in hyper- (robust) and hypo- (asthenia) functioning patients with first- and second-grade amenorrhea. We observed no significant differences in the rate of change of these hormones between hyper- and hypo-functioning patients. Ovulation occurred in 61.3% and 66.7% of patients with first-grade amenorrhea, and in 27.3% and 22.4% of patients with second-grade amenorrhea, respectively. No significant difference was observed in the ovulation rate after an 8-week treatment with Unkei-to between hyper- and hypo-functioning patients.

These results indicate that Unkei-to is effective in improving gonadotropin and estradiol secretion in the treatment of either hyper- or hypo-functioning anovulatory women.

Keywords: Unkei-to; Anovulatory Women; Hyper-functioning Condition; Hypo-functioning Condition; Endocrine Changes.

Introduction

Over time, traditional Oriental-Asiatic medicine has developed a system seemingly different from that of modern Western medicine. Kampo medicine (Chinese medicine) has been reported to be effective in treatment of menstrual and climacteric disorders. Unkei-to is composed of 12 herbs (Table 1) and known to be effective for various menstrual disorders, abnormal uterine bleeding and infertility (Igarashi, 1985). Its mechanism(s) of action, however, is not yet fully understood. In experiments on cultured rat pituitary cells and sequential

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Table 1. Composition of Unkei-to

Unkei-to Formula orbis tepefactum (more Yang)		
Components		
(1)	Bakumondo = Ophiopogonis tuber (slightly Yin)	4.0 g
(2)	Hange = Pinellae tuber (more Yang)	4.0 g
(3)	Toki = Angelicae radix (neutral)	3.0 g
(4)	Kanzo = Glycyrrhizae radix (neutral)	2.0 g
(5)	Keihi = Cinnamomi cortex (more Yang)	2.0 g
(6)	Shakuyaku = Paeoniae radix (slightly Yin)	2.0 g
(7)	Senkyu = Cnidii rhizoma (more Yang)	2.0 g
(8)	Ninjin = Ginseng radix (slightly Yang)	2.0 g
(9)	Botanpi = Mountain radix (slightly Yin)	2.0 g
(10)	Goshuyu = Evodiae frutus (extremely Yang)	1.0 g
(11)	Shokyo = Zingiberis rhizoma (slightly Yang)	1.0 g
(12)	Akyo = Asini gelatum (neutral)	2.0 g

double-chamber perfusion system of mediobasal hypothalamus and pituitary, Unkei-to stimulated the synthesis and release of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Taketani *et al*., 1988; Aono *et al*., 1988). In humans, we demonstrated that Unkei-to enhances pituitary response to GnRH and improves pulsatile secretion of FSH and LH, resulting in elevation of these gonadotropins and estradiol levels and induction of ovulation in unovulatory young women (Ushiroyama *et al*., 1995). Furthermore, Unkei-to has been reported to induce a significant decrease in the plasma LH level in unovulatory patients with high plasma LH concentration including polycystic ovarian syndrome (PCO) (Ushiroyama *et al*., 2001).

Sho, a holistic pattern of symptoms, is used as the basis for treatment in Kampo medicine. Sho is defined as the process of obtaining information about physical and psychological conditions using six parameters: Ki and body fluids, Yin and Yang, deficiency (asthenia, hypo-function) and excess (robust, hyper-function), exterior and interior, cold and heat, and the five organs. The choice of corresponding prescription for patients depends on information obtained from Sho, together with the particular pathological symptoms provided by patients, and with the results obtained by four diagnostic procedures (observing, listening, questioning and palpating).

Unkei-to is known to be effective for patients with a hypo-functioning condition. Therefore, Unkei-to is considered less effective for patients with hyper-functioning condition on the basis of theoretical concepts of Kampo medicine. To our knowledge, the efficacy of Unkei-to in hyper-functioning unovulatory women has not been reported.

This study aims to (1) compare the efficacy of Unkei-to in hypo-functioning and hyper-functioning patients with anovulatory cycles; (2) to establish the efficacy of Unkei-to in hyper-functioning patients; and (3) analyze the changes in endocrinological status with Unkei-to treatment.

Subjects and Methods

Patients

One hundred and fifty-seven women with hypothalamic amenorrhea (115 patients: hypo-functioning condition; 42 patients: hyper-functioning condition) were studied after informed consent. Ninety-seven and sixty women aged between 17 and 29 years were diagnosed with first- and second-grade amenorrhea after administration of progestogen.

The criteria for inclusion of patients in our studies were: (1) disappearance of menstruation over 6 months before the study with no evidence of pregnancy; (2) low levels of estradiol and progesterone assayed in at least three different samples collected over a range of 60 days before the study; (3) plasma levels of cortisol, testosterone, thyroid stimulating hormone and prolactin within the normal range; (4) normal computerized tomography examination of the sella turcica; and (5) no psychiatric disease. Weight loss between 1 to 4 months before the disappearance of menstruation was observed in 47 patients. The weight of these patients was less than ideal body weight. These reductions ranged between 9.2% and 27.5%, with a mean of 22.5%.

Composition and Preparation of Unkei-to

Unkei-to is composed of 11 herbal drugs and one preparation derived from animal material (aquus gelatin) (Table 1). A mixture consisting of these chopped ingredients as listed in Table 1 was extracted with 1 liter of hot water, condensed with boiling to 600 ml and filtered, lyophilized to prepare lyophilized powder (daily dose of 5 g). The lyophilized powder, which must be stored at 4°C, is processed to obtain granules for commercial preparation (daily dose of 7.5 g containing 2.5 g of drug additives, i.e. lactose) (Tsumura Pharmaceutical Co. Ltd., Tokyo, Japan).

Protocol

One hundred and fifty-seven patients were treated with Unkei-to after withdrawal bleeding. They received 7.5 g of extracted granules of Unkei-to each day (dissolved 2.5 g of the commercial preparation in 100 ml of hot water to take about 30 minutes before every meal) for 12 weeks.

Blood samples were obtained at the beginning and after 4 and 8 weeks of administration of Unkei-to through an indwelling i.v. line at 09:00 hours for analyses of FSH, LH and estradiol. The plasma was immediately separated and frozen after sampling for determination of hormone concentrations. Ovulation rate was assessed after 12 weeks of treatment. Confirmation of ovulation was performed by the disappearance of developed follicles and elevation of basal body temperature from the start of administration of Unkei-to to 12 weeks of treatment.

Hormone Assays

Plasma FSH and LH concentrations were measured by an established radioimmunometric assay with RIA kits (SPAC-S: Daiichi Isotope Laboratory, Tokyo, Japan). Sensitivity of the methods was 0.1 mIU/ml for FSH and LH, intraassay variabilities were 4.0 and 5.3%, and interassay variabilities were 4.5 and 6.0%, respectively. LH intraassay variabilities at different levels were: 9.2% for LH values of 5 mIU/ml, and 4.0% for LH values of 20 mIU/ml. Plasma estradiol was measured with a commercially available radioimmunoassay (Estradiol Direct Radioimmunoassay Kit, Sorin Biomedica, France) as previously described (Ushiroyama *et al*., 1989). Limit of detectability of the assay was 4.5 pg/ml, and intra- and interassay variabilities were 6.0 and 9.0%, respectively. Cross-reactivity of estrone with the estradiol kit was less than 0.7%.

Data Analysis

Results were evaluated using the Wilcoxon-matched pairs signed-rank test. A p value of less than 0.05 was considered significant.

Results

The demographic data are displayed in Table 2. There were no significant differences in age, duration of amenorrhea, plasma LH or prolactin concentrations. However, we observed significant differences in BMI ($p < 0.0001$), plasma FSH ($p < 0.01$) and estradiol ($p < 0.0001$) concentrations between the hyper- and hypo-functioning groups.

Effects of Unkei-to on Endocrinological Condition

Figure 1 shows changes in plasma levels of FSH, LH and estradiol during Unkei-to administration.

Table 2. Patient Characteristics (Mean ± SD; Range)

	Patients of Hyper-functioning Condition	Patients of Hypo-functioning Condition	p
Enrolled (n)	42	115	
Age (Year)	23.8 ± 4.08	24.0 ± 4.11	NS
Duration of Amenorrhea (Month)	10.5 ± 7.5	9.8 ± 7.2	NS
BMI (kg/m^2)	25.3 ± 0.8	22.8 ± 0.9	$p < 0.0001$
Endocrinological Condition			
FSH (mIU/ml)	4.2 ± 0.73	3.8 ± 0.81	$p < 0.01$
LH (mIU/ml)	2.2 ± 0.69	2.4 ± 0.82	NS
Estradiol (pg/ml)	18.6 ± 7.88	12.4 ± 7.75	$p < 0.0001$
Prolactin (ng/ml)	4.3 ± 2.35	4.6 ± 2.42	NS

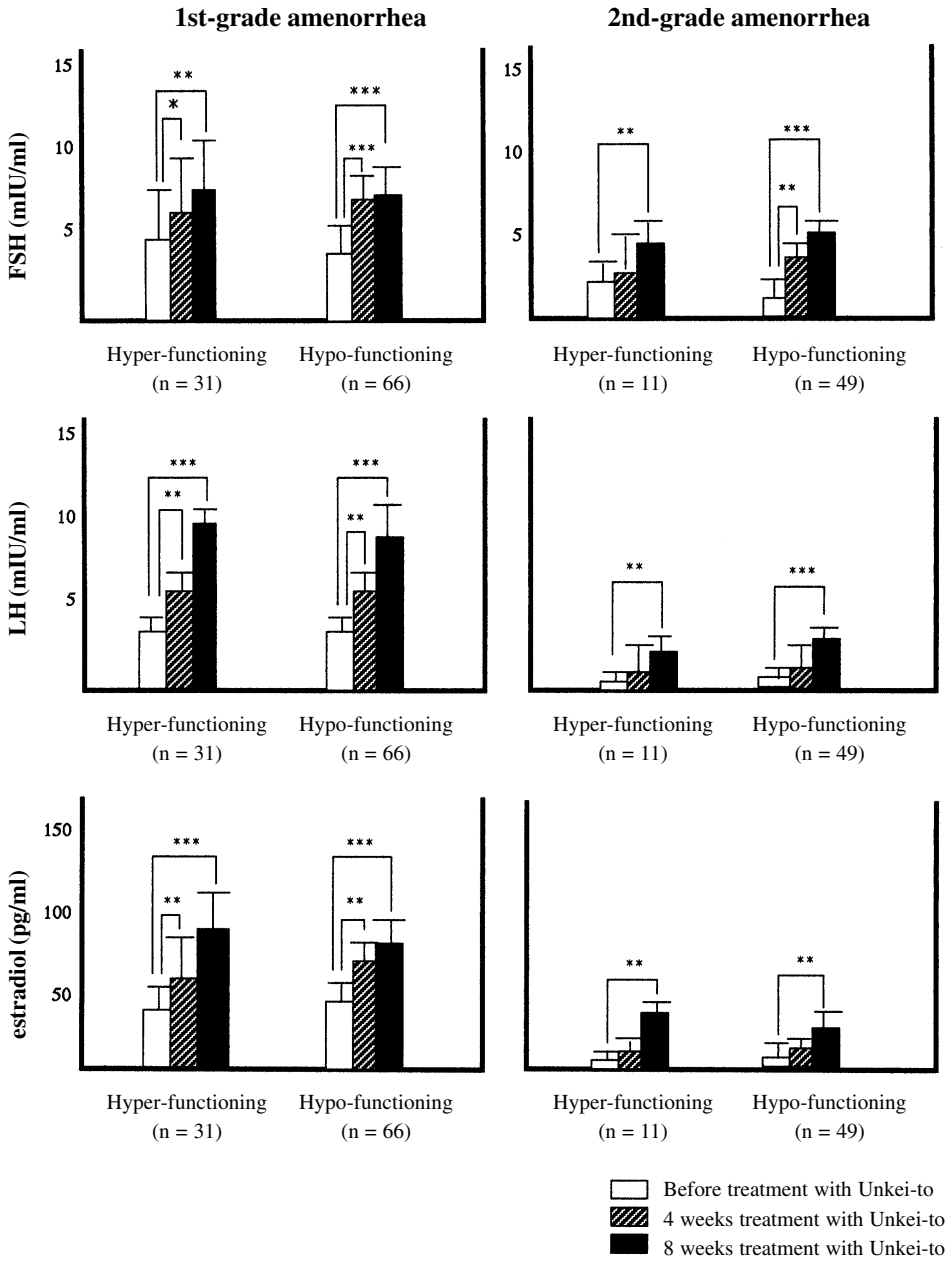

Figure 1. Changes of mean blood levels of FSH, LH and estradiol during treatment with Unkei-to. Left: anovulatory patients with first-grade amenorrhea. Right: anovulatory patients with second-grade amenorrhea. Open column: basal levels before treatment with Unkei-to. Shadowed column: levels after 4-week treatment. Closed column: levels after 8-week treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

In hyper-functioning patients with first-grade amenorrhea, the plasma levels of hormones increased significantly (FSH: 1.33-fold, $p < 0.05$; LH: 1.63-fold, $p < 0.01$; estradiol: 1.40-fold, $p < 0.01$) at 4 weeks compared with those before administration. Eight-week administration of Unkei-to further increased the level of each hormone significantly (FSH: 1.50-fold, $p < 0.01$; LH: 2.80-fold, $p < 0.001$; estradiol: 2.07-fold, $p < 0.001$) .

In hypo-functioning patients with first-grade amenorrhea, the plasma levels of FSH (1.67-fold, $p < 0.001$), LH (1.65-fold; $p < 0.01$) and estradiol (1.56-fold; $p < 0.01$) also increased significantly at 4 weeks compared with before administration. After 8 weeks of treatment, the mean plasma values of FSH (1.84-fold), LH (2.33-fold) and estradiol (1.73-fold) were significantly elevated ($p < 0.001$). No significant difference in these hormones was detected between hyper- and hypo-functioning patients after 8-week treatment with Unkei-to.

On the other hand, in hyper-functioning patients with second-grade amenorrhea, plasma FSH, LH and estradiol levels tended to increase after 4 weeks of treatment with Unkei-to, although not to a significant extent because of wide variations among individual patients. However, at 8 weeks these hormone levels were increased (FSH: 1.92-fold, LH: 5.51-fold, estradiol: 7.0-fold) significantly ($p < 0.01$).

In comparison, hypo-functioning patients with second-grade amenorrhea exhibited significantly elevated FSH levels (2.90- and 4.15-fold) at 4 and 8 weeks ($p < 0.01$ and $p < 0.001$, respectively) above baseline values. After 8-week treatment of hypo-functioning patients with second-grade amenorrhea, plasma LH and estradiol concentrations were markedly increased 19.0 and 12.5 times, respectively ($p < 0.001$ and $p < 0.01$).

After 8-week treatment with Unkei-to for second-grade amenorrhea, the change of plasma FSH from baseline exhibited no significant difference nor was there any significant change in plasma LH or estradiol levels between hyper- and hypo-functioning patients.

Effects of Unkei-to on the Ovulation

Table 3 shows the endocrinological outcomes and the effect on ovulation with Unkei-to administration.

Among 31 hyper-functioning patients with first-grade amenorrhea, improvements of plasma FSH, LH and estradiol levels were observed in 71.0% (22/31), 51.6% (16/31) and 80.6% (25/31) of patients, respectively, based on an increase of 50% or more above basal level.

Among 66 hypo-functioning patients with first-grade amenorrhea, improvements of these hormone levels were also observed in 69.7% (46/66), 56.1% (37/66) and 86.4% (57/66) of patients, respectively.

On the other hand, among 11 hyper-functioning patients with second-grade amenorrhea, improvements of plasma FSH, LH and estradiol levels with administration of Unkei-to were observed in 72.7% (8/11), 54.5% (6/11) and 36.4% (4/11) of patients, respectively.

Among 49 hypo-functioning patients with second-grade amenorrhea, improvements of these hormone levels were observed in 75.5% (37/49), 63.3% (31/49) and 34.7% (17/49) of patients, respectively.

Table 3. Improvement of Endocrine Status and Achievement Rate of Ovulation after 8-week treatment with Unkei-to

	Improvement of FSH Secretion		Improvement of LH Secretion		Improvement of Estradiol Secretion		Achievement of Ovulation	
	No.	%	No.	%	No.	%	No.	%
First-Grade Amenorrhea (n = 97)								
Hyper-functioning	22/31	71.0	16/31	51.6	25/31	80.6	19/31	61.3
Hypo-functioning	46/66	69.7	37/66	56.1	57/66	86.4	44/66	66.7
Significance	NS	NS	NS	NS	NS	NS	NS	NS
Second-Grade Amenorrhea (n = 60)								
Hyper-functioning	8/11	72.7	6/11	54.5	4/11	36.4	3/11	27.3
Hypo-functioning	37/49	75.5	31/49	63.3	17/49	34.7	11/49	22.4
Significance	NS	NS	NS	NS	NS	NS	NS	NS
Total (n = 157)								
Hyper-functioning	30/42	71.4	22/42	52.4	29/42	69.0	22/42	52.4
Hypo-functioning	83/115	72.2	68/115	59.1	74/115	64.3	55/115	47.8
Significance	NS	NS	NS	NS	NS	NS	NS	NS

NS = No significant difference was observed between the hyper- and hypo-functioning groups.

Unkei-to induced ovulation in 61.3% (19/31) and 66.7% (44/66) of hyper- and hypo-functioning anovulatory patients with first-grade amenorrhea, respectively. In second-grade amenorrhea, we observed ovulation induction rate of 27.3% (3/11) and 22.4% (11/49) in anovulatory patients of hyper- and hypo-functioning conditions, respectively. No significant difference in the ovulation rate after 8-week treatment with Unkei-to was observed between hyper- and hypo-functioning patients.

No major complications or adverse effects occurred during administration of Unkei-to.

Discussion

Treatment for secondary amenorrhea in adolescent girls and young women is required to ensure future fertility. Such treatment, however, is not easy since steroid hormones and strong stimulators of ovulation are not recommended due to several side effects, and young women, especially teenagers, appear to be reluctant to visit gynecology clinics.

A number of herbal (Kampo) medicines have been used for many centuries in China and Japan for the treatment of menstrual disorders and infertility (Igarashi, 1985; Aono *et al*., 1988; Takahashi and Kitao, 1994; Ushiroyama *et al*., 1995 and 2002). In general, traditional Chinese herbal prescriptions are rather inexpensive and safe with few side effects, and have restored biological balance. As previously reported, Toki-shakuyaku-san (TJ-23) has a neuroendocrine effect on ovulation in amenorrheic patients (Hagino and Koyama, 1988), while Shakuyaku-kanzo-to (TJ-68) decreases the serum free-testosterone level in patients with polycystic ovarian disease (Takahashi and Kitao, 1994) and stimulates pituitary dopamine receptors (Fukushima and Ota, 1988) resulting in a reduction of serum prolactin levels in

hyperprolactinemic patients. On the other hand, Unkei-to stimulates the synthesis and release of gonadotropins in rat pituitary (Taketani *et al*., 1988; Aono *et al*., 1988). Recent studies have revealed the mechanism by which Unkei-to regulates the diencephalic-pituitary-ovarian axis in humans (Ushiroyama *et al*., 1995 and 2001).

The identification of pathological status based on the pathophysiological concepts of Kampo (Chinese herbal) medicine is unique. The importance for Kampo prescriptions of knowing particular pathological characteristics was presented earlier. Sho refers to the disease status to which a Kampo formula is applied and is expressed in terms of the Kampo formula to be administered. Sho does not necessarily correspond to a Western medical disease as it is an expression of disease status determined by assessing the pattern of symptoms present, the stage of the disease, and the degree of stamina (hyper-functioning, hypo-functioning, etc.) In daily practice, Sho is determined by recording subjective symptoms and body signs (Terasawa, 1993). Unkei-to has been used for hypo-functioning patients based on traditional Kampo examination.

In this study, we investigated the effects of Unkei-to on anovulatory hyper- and hypo-functioning patients. We also observed their increases in plasma FSH, LH and estradiol levels in an 8-week treatment, without significant differences between the groups. These results show that Unkei-to can be used in treatment without performing unique traditional Sho diagnosis. In patients with hypophyseal ovulation disorders not included in Kampo medicine, Unkei-to treatment stimulated the hypophysical-pituitary-ovarian axis, indicating that Kampo examination can be omitted in patients with functional amenorrhea, and can be expected to have a clinical effect comparable to that of treatment with Unkei-to based on Sho diagnosis.

Several authors have reported high ovulatory rates in patients with hypothalamic amenorrhea treated with pulsatile GnRH (Brinjer *et al*., 1985), hMG-hCG (Fleming *et al*., 1985), or clomiphene citrate (Jewelewicz, 1975). However, these treatments are expensive and frequently produce complications (Schenker and Nabot, 1987). We obtained ovulatory rates of 52.4% and 47.8%, respectively, in hyper- and hypo-functioning anovulatory patients treated with Unkei-to for 8 weeks, and observed no major complication or side effects. In addition, Unkei-to is relatively inexpensive.

We conclude that Unkei-to is effective as a traditional herbal medicine for normalization of plasma gonadotropin and estrogen levels, and achieving ovulation in anovulatory young women with differing patterns of Sho based on unique traditional Kampo examination.

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