Effects of Hokoei-to (Pu-gong-ying-tang), Kampo formula, on estradiol and progesterone contents in brain regions and serum in ovariectomized mice

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Abstract

We investigated the effect of the Hokoei-to on the estradiol and progesterone contents of ovariectomized mice. The Hokoei-to was decocted from crude drugs, and freeze-dry extracts were prepared. The experimental mice were given the Hokoei-to extract (240 mg/kg body weight/day) for 20 days after ovariectomy at 7 weeks of age. Contents of estradiol and progesterone in brain tissues and serum were determined by enzyme immunoassay. Hokoei-to increased the levels of estradiol and progesterone in the brain tissues, and tended to increased the levels of estradiol but not progesterone in the serum of ovariectomized mice. At the climacteric, mental and physical disorders develop with the diminished release of sexual hormones, and the Hokoei-to may have a regulatory effect to these hormones.

Key words Hokoei-to (蒲公英湯, Pu-gong-ying-tang), estradiol, progesterone, brain regions, ovariectomized mouse.

Introduction

In females about 50 years old, climacteric disorders such as depression, agitation, sleeplessness, hot flushes, perspiration, and paresthesia are often observed.[1,2] It is also known that psychoneurotic symptoms such as memory [3] and the motor system disorders [4] are found frequently in climacteric women. Estrogen replacement therapy is mainly applied for treatment of these climacteric disorders,[5] it can improve the autonomic nervous system dysfunction such as hot flushes and perspiration, but it may not improve the neurologic symptoms. Antianxiety and/or antidepressive drugs are combined for treatment of these symptoms. Furthermore, adverse effects such as development of breast cancer and intermenstrual flow are serious problems. It is considered to be an advantage that Kampo medicine appears to be gentle effect compared with hormonal agents and can be selected according to the constitution of each patient, and more readily used for long-term treatment because of fewer adverse effects. For these reasons, the usefulness and pharmacological actions of Kampo medicine in gynecological disorders have been highly appreciated, and its effectiveness has often been reported.[6,7]

The Hokoei-to is composed of five crude drugs of *Taraxaci Herba* (蒲公英, Pu-gong-ying), *Angelicae Radix* (当帰, Dang-gui), *Cyperi Rhizoma* (香附子, Xiang-fu-zi), *Moutan Cortex* (牡丹皮, Mu-dan-pi), and *Discoreae Rhizoma* (山薬, Shan-yao) ; it has the effects of resolving swelling and detoxication, and can be used for lack of lactation and mammary swelling. Traditionally, *Taraxaci Herba* has been used for mastopathy, *Cyperi Rhizoma* for regulating menstruation and regulating vital energy, *Moutan Cortex* for

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activating blood circulation to eliminate blood stasis, *Angelicae Radix* for tonifying blood and regulating menstruation, and *Discoreae Rhizoma* for its tonifying effect. *Angelicae Radix* has the effect of promoting uterine contraction, suppressing central nervous system dysfunction, and immuno-activation,[8,9] and *Cyperi Rhizoma* has the effect of inhibiting the biosynthesis of prostaglandin.[10] It is also reported that Hokoei-to has the effect of improving cholesterinemia and increasing the release of estrogen,[11] and regulating the endocrine system in pregnant and ovariectomized rats.[12,13] Furthermore, a lactagogue effect of Hokoei-to in human has been reported.[14] We previously found that the Hokoei-to had the effect of improving the metabolic turnover of dopamine, in ovariectomized mice (unpublished data). These results suggest that the Hokoei-to may be useful for the unidentified clinical climacteric syndrome and climacteric-like symptoms after gynecological surgery. In the present study, we examined the effect of the Hokoei-to on intracerebral steroid hormones.

Materials and Methods

Preparation of Hokoei-to: The Hokoei-to was prepared according to the instructions in the text book.[15] The one day dose contains 8.0 g of *Taraxaci Radix* (Taraxacum officinale Weber), 6.0 g of *Angelicae Radix*, 3.0 g of *Cyperi Rhizoma*, 3.0 g of *Moutan Cortex*, and 4.0 g of *Discoreae Rhizoma*. We obtained German *Taraxaci Radix*, Japanese *Angelicae Radix*, and Chinese *Cyperi Rhizoma*, *Moutan Cortex*, and *Discoreae Rhizoma* from Uchida Wakan-yaku Inc. The five crude drugs were decocted with 540 ml of distilled water for the one-day dose until the contents were reduced by half, and filtered after centrifugation. The filtrate was concentrated to an extract of the Hokoei-to by decompression. The yield was 12.5 % from original herb weight.

Animals and Treatment: Female C57BL/6 mice were obtained from Nihon SLC Inc. at 6 weeks of age, and used after one week of preliminary breeding under 12 hours light and shade cycle at 24-26°C. They were freely fed with CE20 (Clea Japan Inc.). The estrous cycle of each mouse was determined by vaginal smears, and mice in anestrus were selected for this experiment. All the ovaries of the experimental mice were removed under pentobarbital sodium (Nembutal). Sham operations were done for the control mice (Sham group). The extract of Hokoei-to was dissolved in distilled water, and both Sham (Sham-HKT groups) and OVX (OVX-HKT) groups were administered 240 mg/kg/day by feed bottle from the day of operation. The control groups (Sham-H_2O, OVX-H_2O) were given only water. At 20 days after administration, the blood was collected from the trunk, allowed to clot for 1 hour and centrifuged at 1000×g for 15 min at 4°C. Serum was preserved at -80°C. The brains were extirpated and preserved at -80°C. The thymus, spleen, and adrenal glands were removed and their weights were measured.

Sectioning of brains: The brains were sectioned to cerebral cortex (CC), ventral hippocampus (VH), and dorsal hippocampus (DH) on dry ice by the Glowinski & Iversen method.[16]

Measurement of steroid hormones: Phosphate buffer saline (PBS^-) was added to each section of brain tissue, which was homogenated ultrasonically at 4°C for 30 seconds after measurement of the weight, and centrifuged at 15,000×g for 15 min at 4°C. The supernatant of each section was taken. The contents of estradiol and progesterone in the supernatant were determined by using Enzyme Immunoassay kit (Cayman Chemical company, MI, USA). Those in the serum were also examined by the ELISA kit.

Statistics: Statistical significance of difference of means were performed by ANOVA followed by Dunnett's post hoc procedure.

Results

Effects of Hokoei-to on the weights of organs

We found an increase in the weights of spleen and a tendency to increase in thymus, and a tendency to decrease in adrenals in the ovariectomized mice (Table I). However, there were no difference in the groups treated with Hokoei-to.

Effect of Hokoei-to on estradiol content in the brain tissues

The contents of estradiol in brain tissues are shown in Figure 1. In the CC, the content was 53.6± 6.55 pg/mg in the Sham-H_2O group, and 50.7±4.54

Table 1 Effects of Hokoei-to (HKT) on the weight of spleen, thymus, and adrenal in ovariectomized mice.

	Spleen (mg/g Bwt)	Thymus (mg/g Bwt)	Adrenal (mg/g Bwt)
Sham+H_2O	3.16±0.03	3.58±0.05	0.34±0.04
Sham+HKT	3.06±0.22	3.05±0.32	0.30±0.03
OVX+H_2O	3.92±0.14**	4.19±0.32	0.23±0.02
OVX+HKT	3.65±0.23	4.10±0.20	0.22±0.01

Female C57BL/6 mice were ovariectomized (OVX) at 7 weeks of age, the control group mice were sham operated. Sham-HKT and OVX-HKT groups were given HKT (240 mg/kg body weight) daily in drinking water for 20 days. Sham-H_2O and OVX-H_2O groups were given drinking water. Each value represents the mean±S.E. of 5-6 mice. The differance vs Sham-H_2O mice is indicated. **: $p<0.01$.

pg/mg in the OVX-H_2O group. We thus could not find any difference after ovariectomy. Estradiol was 63.0± 2.44 pg/mg in the Sham-HKT group, which was higher compared with the Sham control group, but was not significant. In the OVX-HKT group, the contents of estradiol was 66.0±4.16 pg/mg and was observed to increase significantly compared with the OVX-control group ($p<0.05$).

In the DH, the content of estradiol was 57.8±3.21 pg/mg in the OVX-H_2O group, showing a the significant decrease ($p<0.05$) after ovariectomy , while was 74.5±10.3 pg/mg in the Sham-H_2O group. In the groups treated with Hokoei-to, we could not find any effect in the Sham-HKT group, but the OVX-HKT group (79.5±4.64 pg/mg) was the same as the Sham-H_2O group. In the VH, the content of estradiol was 284.2±20.1 pg/mg in OVX-HKT, showing a significant increase (Fig. 1), compared with 204.3±21.6 pg/mg in the OVX-H_2O group.

Effect of Hokoei-to on progesterone content in the brain tissues

The contents of progesterone in the brain tissues are shown in Fig. 2. In the CC, the content was 2.14± 0.22 ng/mg in the Sham-H_2O group, and 1.74±0.08 ng/mg in the OVX-H_2O group, showing a significant decrease after ovariectomy ($p<0.05$). A content of 2.75±0.32 ng/mg was observed in the Sham-HKT group , which showed a tendency to increase compared with the Sham-H_2O group. In the OVX-HKT group, 2.04±0.09 ng/mg was observed, a significant increase compared with the OVX-H_2O group ($p<$ 0.01). In the DH, progesterone content was 2.67±0.17

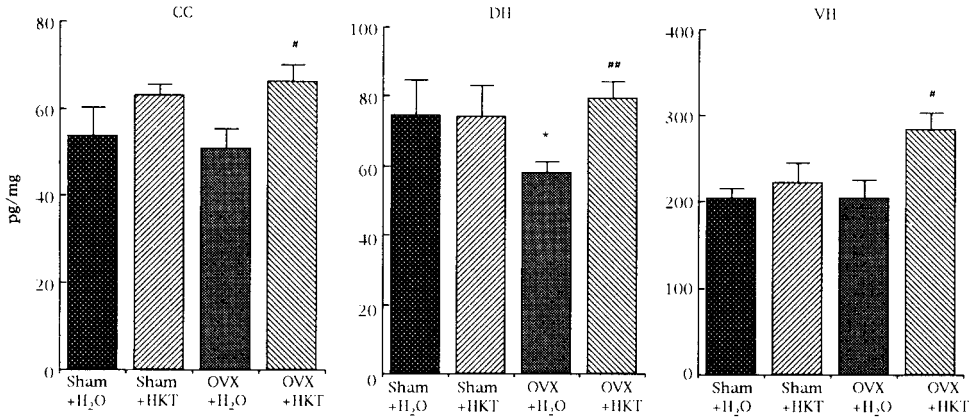

Fig. 1 Effects of Hokoei-to (HKT) on estradiol contents of cerebral cortex (CC), dorsal hippocampus (DH), and ventral hippocampus (VH) in ovariectomized (OVX) mice.
Female C57BL/6 mice were ovariectomized (OVX) at 7 weeks of age, the control group mice were sham operated. Sham-HKT and OVX-HKT groups were given HKT (240 mg/kg body weight) daily in drinking water for 20 days. Sham-H_2O and OVX-H_2O groups were given drinking water. Each column represents the mean ± S.E. of 5-6 mice. Significantly different from Sham-H_2O group at * $p<0.05$. Significantly different from OVX-H_2O group at # $p<0.05$, ## $p<0.01$.

ng/mg in the Sham-H_2O group, and 1.98±0.12 ng/mg in the OVX-H_2O group, showing a significant decrease after ovariectomy ($p<0.01$). In the Sham-HKT group, 3.88±0.93 ng/mg was observed, which was a high value but not significant compared with Sham-H_2O group. On the other hand, the content was 2.98±0.14 ng/mg in the OVX-HKT group, significantly increased compared with the OVX-H_2O group ($p<$

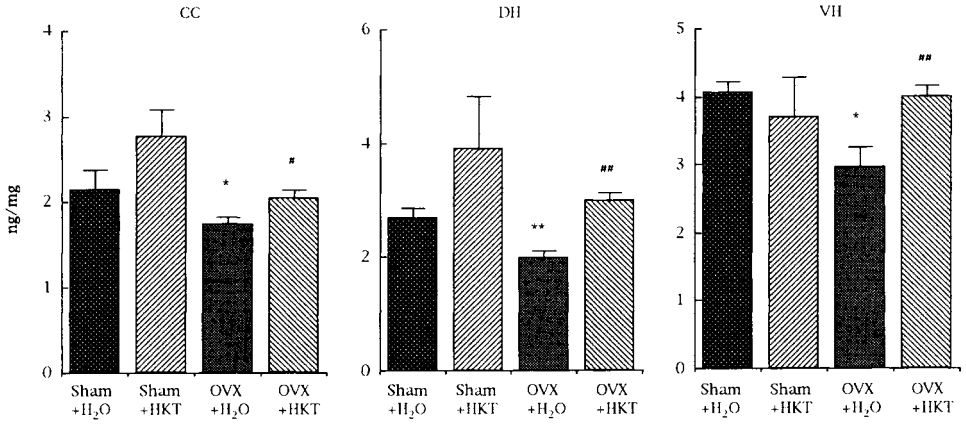

Fig. 2 Effects of Hokoei-to (HKT) on progesterone contents of cerebral cortex (CC), dorsal hippocampus (DH), and ventral hippocampus (VH) in ovariectomized (OVX) mice.
Female C57BL/6 mice were ovariectomized (OVX) at 7 weeks of age, the control group mice were sham operated. Sham-HKT and OVX-HKT groups were given HKT (240 mg/kg body weight) daily in drinking water for 20 days. Sham-H_2O and OVX-H_2O groups were given drinking water. Each column represents the mean±S.E. of 5-6 mice. Significantly different from Sham-H_2O group at $^{**}p<0.01$, $^{*}p<0.05$. Significantly different from OVX-H_2O group at $\#p<0.05$, $\#\#p<0.01$.

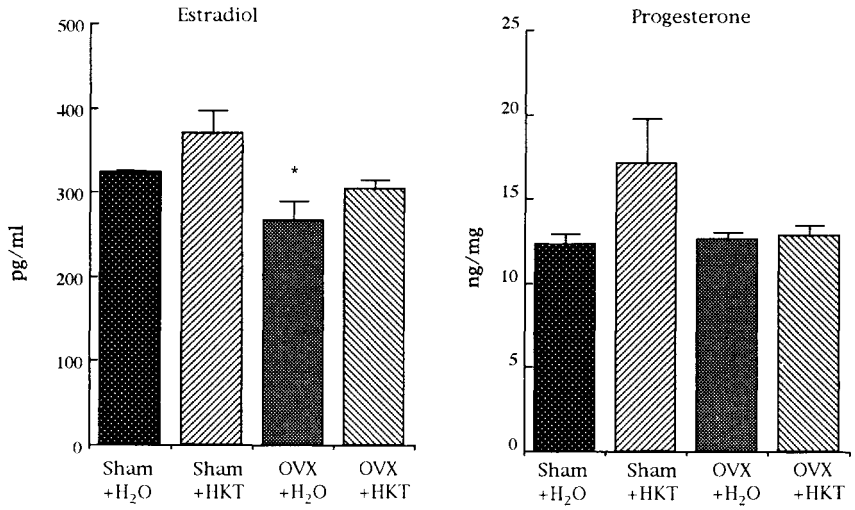

Fig. 3 Effects of Hokoei-to (HKT) on serum estradiol and progesterone level in ovariectomized (OVX) mice.
Female C57BL/6 mice were ovariectomized (OVX) at 7 weeks of age ; the control group mice were sham operated. Sham-HKT and OVX-HKT groups were given HKT (240 mg/kg body weight) daily in drinking water for 20 days. Sham-H_2O and OVX-H_2O groups were given drinking water. Each column represents the mean±S.E. of 5 6 mice. Significantly different from Sham-H_2O group at $^{*}p<0.05$.

0.01), and showed a similar value in the Sham-H_2O group. In the VH, the content was decreased after ovariectomy, and here again the Hokoei-to suppress the decrease in progesterone, giving 3.98±0.17 ng/mg in OVX-HKT group over 2.95±0.30 ng/mg in the OVX-HKT group (Fig. 2).

Effect of Hokoei-to on the serum hormone levels

The serum level of estradiol was 324.2±0.9 pg/ml in the Sham-H_2O group, and decreased to 276.8±9.7 pg/ml in the OVX-H_2O group. The level was 368.7±27.6 pg/ml in the Sham-HKT group, and showed a tendency to increase in the OVX-HKT group compared with the OVX-H_2O group. The serum level of progesterone was 12.2±0.60 ng/ml in the Sham-H_2O group, and increased to 17.0±2.70 ng/ml in the Sham-HKT group. However, there was no difference between the level of progesterone in the OVX-H_2O and OVX-HKT groups (Fig. 3).

Discussion

At the climacteric, neurologic disorders such as memory disturbance[3] may appear with the decrease of estrogen release.[1,2] It has been reported in ovariectomized animals that the release potential of neurotransmitters are decreased in the central nervous system,[17] memory function disorders and[18] lymphocyte transformation are reduced,[19] and endocrine disturbance also appear.[20]

Hokoei-to is composed of five crude drugs which improve cholesteremia and increase estrogen,[11] regulates the endocrine system in pregnant and ovariectomized rats.[12,13] The Hokoei-to is reported to have the clinical effect of promoting lactation.[14] Considering the favorable effects, the Hokoei-to is expected to be of use in treating unidentified complaints and psychoneurotic symptoms in women of menopausal age.

In present experiment, we found that the Hokoei-to tended to reverse the reduction of serum estradiol, but not effected the serum progesterone in ovariectomized mice. It was shown that the effects of Hokoei-to on estradiol and progesterone were different. Since nerve system, immune system and endocrine system are effect mutually. OVX also effect thymus and spleen weight. Changes of cytokines and lymphocytes functions are under investigation.

Estrogen may be involved in both growth and activation of neurons in the central nerves system. The effects of estrogen on neuron formation are in the sexual specificity of behavior, cognitive function, and preference between male and female. The relations of OVX and neurotransmitters have been well investigated,[21,22] but the relations of OVX and sexual hormone in brain are scarcely reported. In present experiment, we found that the administration of Hokoei-to showed an increased estradiol and progesterone not only in the serum, but also in the brain tissues. We investigated the changes of sexual hormones in brain sections and found that the contents of estradiol and progesterone in DH decreased significantly in the ovariectomized mice compared with the sham operated mice. Administration of Hokoei-to therefore can suppress the decrease of estradiol and progesterone in DH, and increase the levels of estradiol and progesterone in CC and VH. Progesterone may stimulate hypothalamic serotonin synthesis.[23] As the mechanisms to restore estrogen and progesterone, we hypothesize that 1) the compensational production from the other organs, 2) enhancement of enterohepatic circulation of hormones. Estrogen can facilitate the acquisition and retention of memory,[24] restore injured neurons,[25] activate cholinergic neurons,[26] stimulate the release of norepinephrine and dopamine in the brains,[27,28] and induce dopamine transporter mRNA in the brains of ovariectomized animals.[20] Furthermore, estrogen receptors colocalize with low-affinity nerve growth factors (NGF) receptor in the cholinergic neurons of the basal forebrain.[29] The Hokoei-to is therefore expected to have the effect remaining neuronal tissue and relieving physical and mental disturbances in climacteric.

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和文抄録

我々は卵巣摘出マウスを用い，脳組織および血清中の

ホルモンに対する蒲公英湯の影響を検討した。蒲公英湯エキスは 240 mg/kg/day となるように手術翌日より 20 日間経口投与した。投与開始後 20 日目に血清を採集し，脳を摘出した。脳の大脳皮質，背部海馬と腹部海馬の分画および血清中の estradiol, progesterone は enzyme immunoassay 法で測定された。その結果，蒲公英湯投与により卵巣摘出マウスの脳組織中のホルモンレベルの低下を抑制することが示された。更年期では精神的，身体的変化が sexual hormone の低下に伴ってみられるが蒲公英湯には sexual hormones を調整する作用が期待された。

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