Effect of Hachimi-jio-gan on adrenal corticosteroids

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Abstract

Hachimi-jio-gan (八味地黄丸, HJ) is a well-known Kampo prescription used for common symptoms accompanying aging and some kinds of illnesses including hypertension, bronchial asthma and diabetes mellitus. HJ is thought to act on neurological, immunological and endocrinological systems with its effect on many symptoms. Its favorable effect on the pituitary-adrenal gland system was demonstrated by an increase in urinary 17-hydroxy corticosteroid (17OHCS) and a decrease in the required dosage of steroids for steroid-dependent asthmatic patients, providing the first evidence of how HJ acts on adrenal corticosteroids. In this study, we examined the effect on the corticosteroid levels of seven healthy volunteers following four weeks of HJ administration in a negative cross-over trial. Serum dehydroepiandrosterone sulfate (DHEAS) significantly increased, the ratio of serum pregnenolone/17α-OH pregnenolone decreased, and the ratio of 17α-OH pregnenolone/dehydroepiandrosterone did not change in comparison with the control. This result suggested that HJ might activate 17α-hydroxylase. The effect of HJ on many symptoms was considered to be partly exhibited through an increase in serum DHEAS.

Key words dehydroepiandrosterone, dehydroepiandrosterone sulfate, Hachimi-jio-gan, 17α-hydroxylase, pregnenolone.

Abbreviations DHEA, dehydroepiandrosterone ; DHEAS, dehydroepiandrosterone sulfate ; HJ, Hachimi-jio-gan ; 17OHCS, 17-hydroxy corticosteroid.

Introduction

Aging and treatment for related symptoms are now problems facing our society. In Chinese and Japanese traditional medicine, aging had been considered as a hypofunction of "Kidney". The concept of "Kidney" comprised not only kidney function but also bone metabolism, brain function, respiratory function, nutrition and even sexual function. Hachimi-jio-gan (八味地黄丸, HJ) is a well-known prescription used for the hypofunction of "Kidney".[1] The traditional indication of HJ was common symptoms with aging such as blurred vision, lumbago, muscle weakness or numbness of leg, impotence and pollakisuria. HJ has also been used for other kinds of illnesses including diabetes mellitus, hypertension and bronchial asthma.

HJ was thought to act on neurological, immunological and endocrinological systems, exerting its effect on many symptoms. A decrease in urinary 17-hydroxy corticosteroid (17OHCS) levels in patients with a type of hypofunction of "Kidney", who had some kinds of illnesses including bronchial asthma, neurasthenia, scleroderma and systemic lupus erythematosus had already been pointed out in the late 1950s by Tianje Gu and his coworkers at the Shanghai Medical College.[2] Following HJ and some Kampo formulas similar to HJ, their urinary 17OHCS levels

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recovered to the normal range while improving their symptoms. In spite of their report, such increasing tendency in urinary 17OHCS levels of our HJ treated patients with bronchial asthma has not always been observed (unpublished observation).

Egashira[3] reported that HJ might have a favorable action on the pituitary-adrenal gland system because this prescription could improve the severity of steroid dependent asthma and decrease the steroid dosage, but the mode of action of HJ on this system has been unknown. The present study was performed to determine in what manner HJ acted on adrenal corticosteroids of healthy volunteers.

Subjects and Methods

Subjects : The subjects were seven male healthy volunteers and their age was 43.0±6.2 (mean±S.D.) years. Informed consent was obtained from each subject.

Substances : HJ prepared by the hospital pharmacy of the Toyama Medical Pharmaceutical University was used in this study. A 1 gm powder of HJ consisted of the following eight crude drugs : The steamed root of *Rehmanniae Radix* 0.36 g, *Poria* 0.13 g, *Corni Fructus* 0.18 g, *Moutan Cortex* 0.13 g, *Dioscoreae Rhizoma* 0.18 g, *Cinnamomi Cortex* 0.04 g, *Alismatis Rhizoma* 0.13 g and *Aconiti Tuber* 0.05 g. One 2 gm pill consisted of 1 gm of this preparation, 0.8 grams of honey and 0.16 ml of Japanese sake. Six pills (12 gm) of it were given daily to each subject orally after meals for four weeks.

Protocol : The subjects were advised to eat, sleep and exercise as usual during this study. To avoid that corticosteroid levels might be influenced by episodes of stress, this study was not held during any period that included three or more successive holidays. The data was excluded when they suffered from any kind of illness (even common cold) from two weeks before beginning of the study to the end of it.

This protocol was planned as a negative cross-over trial. It consisted of two periods, the medication period and the control period. In the medication period, subjects were administered HJ for four weeks and their blood was collected after an overnight fasting at 9 AM of the 1 st, 15 th and 29 th days. All plasma samples had been stored below -60°C until this protocol finished, and used in a single assay for measurements of steroid hormone levels by radioimmunoassay : Cortisol and dehydroepiandrosterone sulfate (DHEAS) were measured in Bio Medical Laboratories, Tokyo, and pregnenolone, 17α-OH pregnenolone, dehydroepiandrosterone (DHEA), progesterone, 17α-OH progesterone and androstenedione were measured in Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo. Intra- and inter-assay precision coefficients of variations for these plasma steroid levels were 5.0 % and 8.7 % for cortisol, 5.0 % and 4.4 % for DHEAS, 14.0 % and 11.4 % for pregnenolone, 10.5 % and 11.3 % for 17α-OH pregnenolone, 2.9 % and 3.0 % for DHEA, 4.7 % and 8.0 % for progesterone, 8.4 % and 8.9 % for 17α-OH progesterone and 4.1 % and 4.1 % for androstenedione.

During the control period they were not adminis-

Table I The indicative criteria for treating symptoms of aging with Hachimi-jio-gan as proposed by Mitsuma *et al.*[4]

Major symptoms
1 Disorders in urination (pollakisuria, hyperuria, oligouria or nocturnal pollakisuria)
2 Feeling cold of the lower extremities or feeling hot of the planta
3 Fatigue, weakness, numbness and pain in the hips and legs
4 Weakness of lower abdominal muscle tension and decreased sensation of lower abdominal skin

Minor symptoms
1 Dry mouth or thirst
2 Edema of the legs
3 Decreased sexual power
4 Visual disturbance (cataract, eye fatigue, blurred vision)
5 Chronic respiratory illness
6 Hard of hearing

Symptoms for exclusion of the indication
Gastro-intestinal symptoms

Indicative criteria
A patient who has two or more major symptoms, or a patient who has one major symptom and two or more minor symptoms.

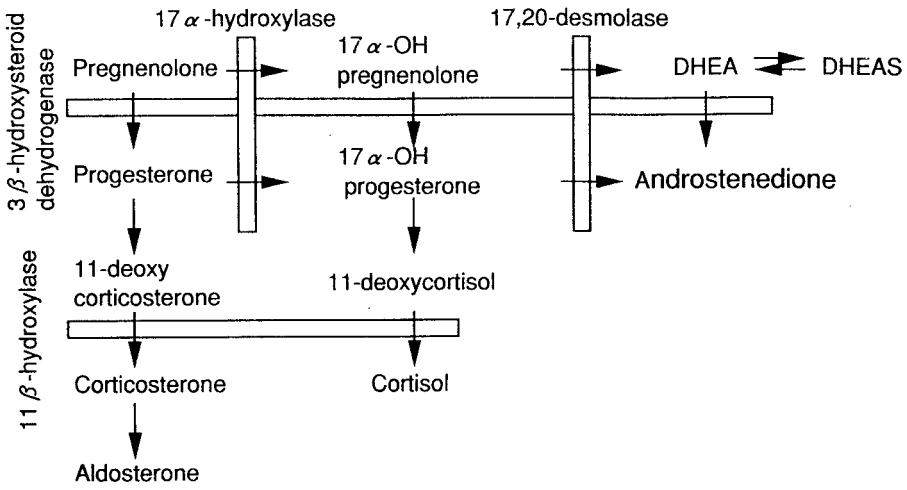

Fig. 1 Human adrenocortical steroidgenic pathways. DHEA, dehydroepiandrosterone ; DHEAS, dehydroepiandrosterone sulfate.

tered any drug and their blood was collected three times, and these steroids levels were measured again. On the days of collecting blood, some symptoms indicated for HJ were checked by the criteria of Mitsuma *et al.*,[4] which are summarized from the traditional indications for HJ in Table I. An interval of a few months with no examinations was set between the two periods. The control period was prior to the medication period in three subjects, and vice versa in the other four subjects. The ratios of the steroid levels were also calculated to analyze the enzymatic activities of the steroidgenic pathway[5] (Fig. 1).

Statistical analysis : Wilcoxon's signed rank test was used for a comparison of these steroid levels between the medication period and the control period at each measurement. All tests were performed at a

Table II Mean (±S.D.) plasma steroid levels (ng/ml) after the administration of Hachimi-jio-gan (HJ). DHEA, dehydroepiandrosterone ; DHEAS, dehydroepiandrosterone sulfate.
d2 W and d4 W, difference between the initial levels and 2W, and 4W (W=week). *$p<0.05$ vs. control (by Wilcoxon's signed rank test).

	Pregnenolone		17α-OH pregnenolone		DHEA		DHEAS	
	Control	HJ	Control	HJ	Control	HJ	Control	HJ
Initial level	0.122±0.083	0.148±0.044	1.98±0.26	2.17±0.57	4.94±1.86	4.67±1.57	2042±486	1938±652
2W	0.133±0.080	0.157±0.081	2.98±0.87	2.95±2.07	5.17±1.64	5.13±2.17	2169±903	2010±833
4W	0.122±0.043	0.094±0.036*	2.14±0.45	2.60±1.09	4.36±1.22	4.64±1.35	1920±395	2179±647
d2W	0.011±0.045	0.009±0.067	0.99±0.92	0.78±2.02	0.23±0.79	0.46±1.51	127±569	72±387
d4W	-0.001±0.060	-0.054±0.044	0.15±0.56	0.43±1.15	-0.59±0.83	-0.03±1.37	-122±272	241±282*
	Progesterone		17α-OH progesterone		Androstenedione		Cortisol	
	Control	HJ	Control	HJ	Control	HJ	Control	HJ
Initial level	0.67±0.08	0.59±0.11	1.81±0.47	1.67±0.53	1.40±0.54	1.56±0.53	101±27	127±47
2W	0.70±0.13	0.57±0.11	2.10±0.72	1.77±0.59	1.84±0.58	1.69±0.89	123±35	116±35
4W	0.64±0.17	0.66±0.19	1.60±0.62	1.53±0.72	1.54±0.50	1.56±0.65	108±36	117±65
d2W	-0.03±0.13	-0.01±0.07	0.29±0.43	0.10±0.65	0.44±0.58	0.13±0.51	-22±27	-11±28
d4W	0.12±0.11	-0.07±0.18	-0.21±0.25	-0.14±0.73	0.14±0.54	0.00±0.43	7±29	-9±40

5 % level of confidence.

Results

The course of the protocol

All subjects were fitted to the indicative criteria for HJ before each period. Following the administration of HJ, three of the seven subjects felt a sense of well-being, and two of them felt a loosening of their leg muscles. Nevertheless, two subjects had mild epigastric discomfort as a side effect of HJ, but both of them could complete this study ; the dosage of HJ was halved for one subject during the latter half of the medication period, and remained unchanged for the other subject. No special changes occurred in their health conditions during the control period, and there were no complications following this study for any of the subjects.

Serum steroids levels (Table II)

All steroids levels were within normal range in both groups, and there was no difference between their initial levels. Pregnenolone decreased significantly as compared to the control level following the oral administration of HJ for four weeks. Simultaneously, serum DHEAS elevated significantly as compared to the control level. Other steroid levels did not change during this study.

Enzymatic activities of the steroidgenic pathway (Table III)

We analyzed the mean (±S.D.) substrate/product ratio in serum steroids as an index of the relative activities of steroidgenic enzymes of the adrenal cortex between the control and HJ-medicated subjects. In reactions with 17α-hydroxylase, the ratio of pregnenolone/17α-OH pregnenolone decreased significantly as compared to the control level after the administration of HJ, but the ratio of progesterone/17α-OH progesterone did not change. In reactions with C17-20 desmolase, neither the ratio of 17α-OH pregnenolone/DHEA nor that of 17α-OH progesterone/androstenedione changed during this study.

Discussion

There were two reasons why we selected middle-aged healthy men (their age was 35 to 53 years) as subjects to study the endocrinological actions of HJ. First, it was very difficult to find subjects more than 60 years old who were not taking some kind of medication. Second, the subjects consisting of men 35 years or more were expected to fit the indicative criteria of HJ. According to our investigation about the frequency of HJ given to outpatients under the concept of traditional Kampo diagnosis,[6] HJ was the

Table III Mean (±S.D.) substrate/product ratio in serum steroids as an index of relative activities of steroidgenic enzymes of the adrenal cortex after administration of Hachimi-jio-gan (HJ).
DHEA, dehydroepiandrosterone ; DHEAS, dehydroepiandrosterone sulfate ; W, week.
*$p<0.05$ vs. control (by Wilcoxon's signed rank test)

1) Reactions with 17α-hydroxylase

	pregnenolone/17α-OH pregnenolone		progesterone/17α-OH progesterone	
	Control	HJ	Control	HJ
Before	0.062±0.043	0.069±0.013	0.39±0.11	0.38±0.13
2W	0.045±0.026	0.059±0.024	0.36±0.09	0.34±0.08
4W	0.056±0.016	0.037±0.007*	0.42±0.10	0.47±0.13

2) Reactions with C17-20 desmolase

	17α-OH pregnenolone/DHEA		17α-OH progesterone/androstenedione	
	Control	HJ	Control	HJ
Before	0.45±0.15	0.49±0.15	1.44±0.57	1.14±0.48
2W	0.58±0.06	0.54±0.13	1.22±0.53	1.14±0.40
4W	0.51±0.10	0.54±0.14	1.12±0.52	1.13±0.66

most commonly prescribed Kampo formula, used by 11 % of all outpatients, and the frequency of HJ increased with age and was higher in men than in women. It was supposed that the actual percentage of patients with indications for HJ was greater than 11 % who were in fact administered, because the traditional indications for HJ were thought to be related to aging.

This might be the first report to describe the endocrinological action of HJ with a control group. This study demonstrated that serum DHEAS increased and pregnenolone decreased after four weeks of HJ administration. Other steroids (17α-OH pregnenolone, 17α-OH progesterone, androstenedione and cortisol) did not change with HJ use.

In spite of the increase in serum DHEAS, serum DHEA did not change. This was considered to be due to differences in their circadian rhythms and in their serum half lives. It was reported that DHEA had stronger activity than DHEAS and was converted from DHEAS by steroid sulfatase which exists in almost all tissues.[5,7] The circadian change within a day in DHEA was similar to the one in cortisol, but that in DHEAS was smaller. Further, the serum half life of DHEA was 25 minutes, much shorter than the 8-11 hours of DHEAS.

DHEAS is one of the adrenal androgens and is regarded as a parameter of aging because it decreases gradually with age after peaking during puberty. It recently became known that DHEAS has anti-glucocorticoid and anti-obesity activity, anti-arteriosclerosis and anti-carcinogenesis effects, work in the prevention of osteoporosis and the regulation of immune competence, and is also active in cellular proliferation and neurological function.[7]

It was reported that HJ improved pain, weakness, numbness and coldness of limbs and waist in aged patients.[8] The actions of HJ resembled actions of DHEA for improving physical changes with aging. Moreover, our results suggest that many actions of HJ may be at least partly shown through the increase in serum DHEAS. As for DHEA, although the reported stronger activity than DHEAS was not observed in the present study, this possibility in HJ also cannot be ruled out.

We have to discuss whether DHEAS might be synthesized from HJ itself, or whether some enzymes might be activated following administration of HJ and then increase DHEAS levels. A drug containing DHEA is commercially available in the United States, and it is made from yam (a generic name of some dioscorea) through some chemical procedures as used for making some steroid drugs. Yam and *Dioscoreae Rhizoma*, one of the medicinal plants composing HJ, belong to the same species of dioscorea. It was reported that three weeks of supplementation of dioscorea, which is a yam steroid extract, could not increase serum DHEAS levels in humans.[9] Considering with this report, it might be possible that *Dioscoreae Rhizoma* together with some or all of the other seven medicinal plants might activate some enzymes for producing DHEAS, in spite of the slight possibility that HJ itself might become DHEAS.

It became clear that 17α-hydroxylase and C17-20 desmolase activated the reaction from pregnenolone to DHEA (Fig. 1). Following the administration of HJ, a decrease in the pregnenolone/17α-OH pregnenolone ratio and no change in the ratio of 17α-OH pregnenolone/DHEA suggested that the enzymatic activity of 17α-hydroxylase might be activated. A decrease in adrenal androgen in elderly people was reported to be derived from a decrease in C17-20 desmolase, because lipofuscin increased in the adrenocortical reticular layer with aging.[10] An increase in the activity of C17-20 desmolase was not observed in our study, and it was supposed that the mean age of 43 years of our subjects might be too young to study this enzymatic activity. A similar study with elderly subjects should be preformed to determine whether HJ activates C17-20 desmolase.

Recently, a new relationship between DHEA and cortisol was revealed,[11] showing that 11β-hydroxylase, which promotes the production of cortisol, was activated following the administration of DHEA to rat (Fig. I). Our study could not determine any effect of HJ on urinary 17OHCS, although the possibility that an increase in serum DHEAS levels following the administration of HJ might raise urinary 17OHCS in postmenopausal asthmatic women whose serum DHEAS was low, was reported.[12]

DHEA and DHEAS are stress-preventing hormones. Details of their regulation system are still

unknown, unlike cortisol and thyroid hormones. It was difficult for us to imagine that the 6-month administration of DHEA did not result in any imbalance of endocrinological homeostasis, as was reported.[13] HJ could be used without fear because neither DHEA nor DHEAS was detected in acetone extracts prepared from powder of HJ by radiommunoassay. A drug containing DHEA should be used only for patients depleted of it. We have given HJ to many aged patients for many years, and adverse effects were rare except for abdominal discomfort and skin itching, that were immediately improved by stopping HJ. HJ was considered to be superior in endocrinological safety.

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和文抄録

八味地黄丸（HJ）は高齢化に伴って出現しやすい様々な症状の治療，あるいは高血圧，気管支喘息，糖尿病などを治療する漢方方剤としてよく知られている。HJのもつこのような多面的な効果の背景には，HJが神経免疫内分泌学系を制御していることが推定される。これまでにHJには尿中17OHCSの上昇やステロイド依存性喘息患者の必要ステロイドの減量により，下垂体副腎系に対する良好な効果のあることが指摘されている。しかし，HJが副腎皮質ホルモン系にどのような影響を与えているかについては知られていない。筆者らは7名の健常者にHJを4週間投与するネガティブ・クロスオーバー試験を行い，血清副腎皮質ホルモン値に対する影響を検討した。その結果，pregnenolone値の低下，dehydroepiandrosterone sulfate（DHEAS）値の上昇を認めた。pregnenolone/17α-OH pregnenolone比の低下および17α-OH pregnenolone/dehydroepiandrosterone比に変化のなかったことより，作用機序として17α-hydroxylaseの活性化が推測された。HJの多面的な効果の一部が血清DHEASを介することが本研究により示唆された。

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