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AN EVIDENCE-BASED STUDY OF KAMPO FORMULA, HACHIMI-JIO-GAN IN TREATMENT OF LOWER URINARY TRACT SYMPTOMS (LUTS)

Hypothesis / aims of study

Kampo medicine is not only widely practiced in Japan, but also integrated into the modern health care system. Hachimi-jio-gan (HJG, *Rehmannia Eight Formula*) is a Kampo formula consisting of eight components (Rehmannia root, Dioscorea and Alisma rhizoma, Aconite tuber, Cinnamon and Moutan bark, Cornus fruit, and Hoelen), which used traditionally for the treatment of various disorders in aging male such as fatigue and lassitude, sexual disorders, dysuria and/or polyuria. Clinically, HJG is currently applied to treat lower urinary tract symptoms (LUTS) of men with benign prostatic hypertrophy (BPH) because of good treatment outcomes, compliance and few side effects. More recently, increasing evidences for involvement of testosterone deficiency in pathogenesis LUTS, metabolic syndrome and erectile dysfunction have suggested that testosterone deficiency provide a pathophysiologic basis for the connections among them (1). On the other hand, estradiol increasing in men after middle age has been implicated in the induction and progression of BPH (2). The conversion of testosterone to estradiol is catalyzed by aromatase. A very recent study found aromatase inhibition increases testosterone and lowers estradiol levels in hypogonadal elder men (3). In this study, the estrogenic and/or androgenic effect of HJG, and aromatase inhibitory activity of each components were examined in MCF-7 cell and enzyme levels. Moreover, the pharmacological studies of HJG on LUTS will be reviewed till now.

Study design, materials and methods

1) The effect of HJG on the growth of human breast cancer cells (MCF-7) was determined by measuring metabolic activity using a 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cell line was obtained from the RIKEN BRC Cell Bank (Japan) and grown in Minimum Essential Medium with 10% (v/v) FBS, penicillin G/streptomycin,1mM sodium pyruvate and 0.1mM non-essential amino acid at 37ºC in a humidified 5% CO_2. When MCF7 cells (3×10^3 cells/well) were planted in 96-well plates under the culture medium 1 day, the cells treated with DMSO (1% v/v, control), ICI 182,780 (10 □M), 17β-estradiol (0.1 □M) and anastrozole (100 □M) dissolved in DMSO or test sample (0.1mg/ml) in PBS for 6 days and the plates were incubated for 3hr by addition of a stock MTT solution (50 ng/well).
2) Aromatase inhibition assay was conducted with CYP19/MFC high throughput inhibitor screening kit (BD Bioscience). Briefly, a cofactor contained an NADPH-regenerating system and control protein (144 □l), and test sample (6 □l) were mixed in a well of a 96-well microplate and warmed for 10 min at 37ºC. The reaction was initiated by adding a pre-warmed enzyme/substrate mix. Reactions were terminated after 30 min by addition of 75 □l stop reagent. In addition, a natural compound chrysin was used as positive control.

Results

1) HJG was evaluated for estrogen and androgen-like effect on MCF-7 cell line treated with 17β-estradiol, estrogen receptor antagonist (ICI 182,780) and aromatase inhibitor (anastrozole). In 17β-estradiol-treated cells, cell viability increased significantly, however, the cell proliferation was blocked by adding ICI 182,780. For comparison, HJG has no effects on the proliferation of MCF-7 with or without ICI 182,780 and anastrozole (Fig.1). To further examine the agonistic effect of HJG on estrogen, the cells were co-cultured with HJG and 17β-estradiol. As a result, there was no effect on cells viability compared with that of the cells only co-cultured with 17β-estradiol.
2) Among the eight components of HJG, the water extracts of Rehmannia root, Cornus fruit and Cinnamon bark showed inhibitory activities concentration-dependently with IC_{50} of 44.7, 52.0, and 59.3 □g/ml, respectively (Table 1).

Interpretation of results

HJG did not show estrogen and androgen-like effects in the MCF-7 cell assay, suggesting that HJG do not directly exert hormonal action per se on LUTS and BPH, but rather via other pathways. And, it also could be explained why HJG is of benefit to the LUTS of patients with BPH in clinical treatment. Furthermore, in the examination of aromatase inhibitory effect, three components, i.e., Rehmannia root, Cornus fruit and Cinnamon bark were found. The results may provide evidences that HJG play a role in increasing androgen and deceasing estrogen productions which may be explained the beneficial effects of HJG on the LUTS/BPH.

Concluding message

Kampo formula is characterized by a mixture of two or more kinds of crude drugs, and studies of individual components are not common. Up to now, HJG has been reported that shows pharmacological actions not only on urinary bladder and urethra, also on micturition reflex. In this study, we found that a part of components of HJG showed aromatase inhibitory activities in addition to HJG per se without estrogen and androgen-like effect. These facts suggest that each component play a part role in pharmacological actions of the formula which may be a multi-target. As this point, further study is under investigation.

Table 1 Inhibitory Activity of the Eight Components of HJG against Aromatase

Test Sample	Final Con. (□g/ml)	Inhibition (%)	IC_{50} value (□g/ml)
	1.22	71.8 ± 13.3	
Chrysin	0.41	61.3 ± 11.0	
(Positive control)	0.14	40.5 ± 11.9	0.28
	0.05	16.6 ± 10.0	

Rehmannia root	100.0 33.3 11.1	100.0 ± 1.2 38.6 ± 4.3 20.6 ± 18.5	44.7
Cornus fruit	100.0 33.3 11.1	55.3 ± 13.1 48.5 ± 11.7 32.6 ± 26.1	52.0
Cinnamon bark	100.0 33.3 11.1	64.0 ± 5.1 32.0 ± 7.6 20.8 ± 12.1	59.3

The values are the means of triplicate experiments.

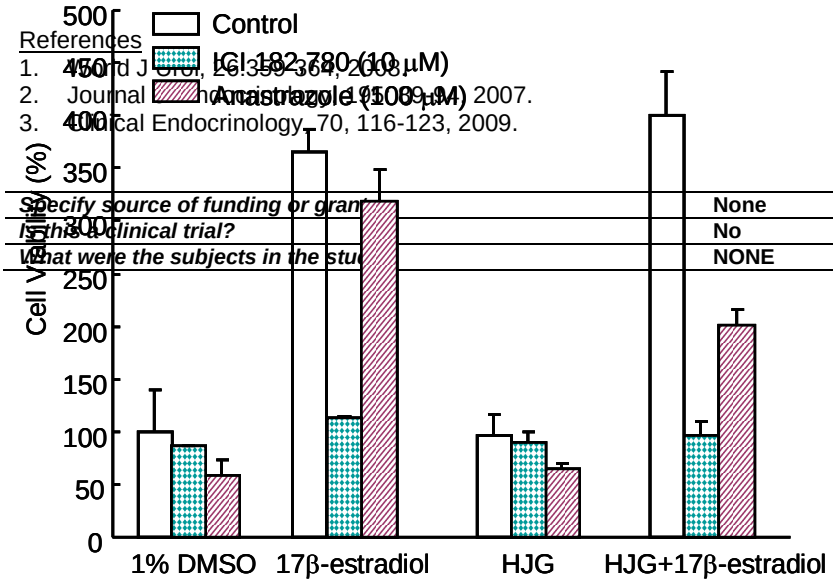

Fig. 1 Effect of HJG on proliferation of MCF-7 cells

References

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Specify source of funding or gran	**None**
Is this a clinical trial?	**No**
What were the subjects in the stu	**NONE**