Herbal Medicine, Hachimi-jio-gan, and Its Component Cinnamomi Cortex Activate the Peroxisome Proliferator-activated Receptor Alpha in Renal Cells

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Abstract. Hachimi-jio-gan is widely used to improve several disorders associated with diabetes, but its mechanism remains poorly understood. In an attempt to clarify the mechanism of Hachimi-jio-gan, we investigated the effects of this herbal medicine and its components in transfection studies of CV1 cells, especially nuclear receptor-mediated actions. One half (0.5) mg/ml of Hachimi-jio-gan activated peroxisome proliferator-activated receptor (PPARα), mediating the activation by 3.1-fold on DR1 response elements; however, it did not affect PPARγ, thyroid hormone receptor, androgen receptor, estrogen receptor or RXR. In addition, this activation was observed in a dose-dependent manner. Next, to determine which components of Hachimi-jio-gan activate PPARα-mediated transcription, 8 of its components (rehmanniae radix, orni fructus, dioscoreae rhizoma, alismatis rhizoma, hoelen, moutan cortex, cinnamomi cortex, aconiti) were tested. Only cinnamomi cortex (1.0 mg/ml) increased PPARα-mediated transcription by 4.1-fold, and this activation was specific for PPARα, and not for other nuclear receptors. Moreover, this PPARα-related activation by cinnamomi cortex is specifically observed in renal cells. Taken together, these findings indicate that Hachimi-jio-gan and cinnamomi cortex may have a pharmacological effect through the target site for PPARα.

Key words: Hachimi-jio-gan, cinnamomi cortex, PPARα

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In Japan and China, traditional herbal medicines (Kampo) have often been used for diabetes mellitus to treat symptoms such as fever, numbness, and edema. In particular, Hachimi-jio-gan is used clinically to improve diabetic nephropathy [1–4]. Recently, Hachimi-jio-gan was reported to suppress hyperglycemia via insulin production and secretion from the pancreas [5]. In addition, Hachimi-jio-gan has been widely used for the treatment of many chronic diseases such as renal nephritis [6], vegetative ataxia [7], major depressive disorder [8, 9], hyperprolactinemia [10], and hyperlipidemia [11]; however, the mechanisms of Hachimi-jio-gan remain unclear.

The objective of this study was to investigate whether Hachimi-jio-gan has functions related to hormonal effects using transfection assays with thyroid hormone receptor, androgen receptor, estrogen receptor, estrogen receptor, RXR, PPARγ, and PPARα into several cell lines. Moreover, Hachimi-jio-gan is composed of eight ingredients, rehmanniae radix, orni fructus, dioscoreae rhizoma, alismatis rhizoma, hoelen, moutan cortex, cinnamomi cortex and aconiti; therefore, we identified which components of Hachimi-jio-gan are responsible for pharmacological effects such as hormonal ligands for nuclear receptors.
Materials and Methods

Cell lines

PC-3 (prostate), HepG2 (liver), and HTB185 (cerebellum) cells were obtained from the American Type Culture Collection (Manassas, VA, USA), COS7 (kidney), and Nthy-ori3-1 (thyroid) was obtained from Dainippon-Sumitomo grown in DMEM, or RPMI1640 supplemented with 10% fetal bovine serum, 0.25 mg/ml streptomycin (GIBCO BRL), and 100 μg/ml penicillin. CV1 cells (kidney) have been described previously (12). Cells were grown on 150 cm² cell culture plates for 48 hours, and then cells were cultured in DMEM containing 10% (v/v) charcoal-stripped FBS in the presence or absence of appropriate ligands. To remove steroid and thyroid hormones, FBS was treated for 24 hours at 4°C with 50 mg/ml of activated charcoal (Sigma) and 30 mg/ml of anion exchange resin (type AGX-8, analytical grade, BioRad).

Transfection

The transfection assays and transfected plasmids were described previously [12]. Human PPARα cDNA was amplified by polymerase chain reaction (PCR) from human liver cDNA. The PCR product was verified as PPARα cDNA by sequencing, and was cloned into the expression vector pKCR2. The PPAR or RXR response elements reporter construct consist two copies of the direct repeat motif of hexamer half sites, TGACCT, spaced by one nucleotide (DR1 element) upstream of the TK109 promoter in the vector pA3Luc (DR1-TKLuc). The positive TRE (thyroid hormone response elements) constructs consist of two copies of a palindromic element upstream of TK109 promoter in the pA3Luc (pal-TKLuc). MMTV-Luc contains the murine mammary tumor virus promoter in pA3Luc. The ERE (estrogen response element)-Luc contains two copies of an ER response element upstream of TK109 promoter in the pA3Luc (pal-TKLuc). The data were quantified as fold luciferase activity, where 1 equals the activity of the reporter in the absence of Hachimi-jio-gan. The transfected receptor expression vectors are indicated below. Results represent the mean ± S.E.M. of three independent experiments.

Results

Hachimi-jio-gan activated PPARα-related transactivation in CV1 cells

Fig. 1 shows the luciferase activities of Hachimi-jio-gan on nuclear receptor in transfected CV1 cells. When 0.5 mg/ml Hachimi-jio-gan was added, luciferase activity increased to 3.1-fold in CV1 cells transfected with the PPARα expression vector and DR1 response element linked to the TK promoter construct; however, Hachimi-jio-gan had no effects on

Dulbecco’s modified Eagle’s medium (DMEM) and sera were purchased from Gibco BRL (Grand Island, NY, USA). Hachimi-jio-gan extract and its component powder were kindly supplied by Tsumura Inc. (Tokyo, Japan). Fenofibrate was kindly supplied by Kaken Inc. (Tokyo, Japan). Before using these herbal components in transfection experiments, those components was added to water, boiled for one hour and filtered.
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thyroid hormone receptor, androgen receptor, estrogen receptor, RXR, and PPARα. These findings suggested that Hachimi-jio-gan might stimulate PPARα specifically.

One component of Hachimi-jio-gan, cinnamom cortex, had a PPARα ligand-like function

Next, to examine which component of Hachimi-jio-gan is responsible for the activation of PPARα, eight gradients of Hachimi-jio-gan were tested in the same system using CV1 cells. As a control, 1 µM fenofibrate caused 7.1-fold activation of TK promoter activity fused to the DR1 element in this system. While 1.0 mg/ml cinnamom cortex enhanced PPARα-mediated activation by 4.1-fold, the other seven gradients did not show any increase of PPARα-mediated transactivation (Fig. 2A). Moreover, stimulation of cinnamom cortex on PPARα was observed in a dose-dependent manner (Fig. 2B). These observations demonstrated that cinnamom cortex, one gradient of Hachimi-jio-gan, functions with a PPAR-α ligand-like effect.

Effects of cinnamom cortex on PPARα were observed specifically in renal cells

To determine whether cinnamom cortex activates PPARα in any cells derived from many kinds of organs, activity was measured in five different cell lines (PC3 (prostate), HepG2 (liver), HTB185 (cerebellum), Nthy-ori3-1 (thyroid), COS7 (kidney)). Cinnamom cortex activated the PPARα-mediated transcription in COS7 cells (2.8-fold) as well as CV1 cells and had no effect in other cells (Fig. 3). These findings suggested that PPARα-mediated effects by cinnamom cortex might be renal-specific.
Discussion

The present study demonstrated that Hachimi-jio-gan activated PPARα induced transactivation. Our study of transfection into CV1 cells clearly showed that Hachimi-jio-gan activated DR1 luciferase activity and this activation is PPARα-specific. Hachimi-jio-gan was reported to have favorable effects on lipid [11] and glucose metabolism [5, 13]; however, this mechanism has not been resolved. Our study suggested one possibility that the effect of Hachimi-jio-gan was induced by PPARα activation.

Moreover, we established that cinnamomi cortex, one component of Hachimi-jio-gan, was mainly responsible for PPARα activation and this activation was dose-dependent with renal tissue-specific activation. Cinnamomi cortex is commonly obtained not only from traditional herbal medicine for treating blood circulation disturbance (14) and inflammatory disease [15], but also from natural food; however, the mechanisms underlying these effects of cinnamomi cortex have yet to be clarified. Only one report revealed that cinnamomi cortex inhibits NF-κB activation and iNOS protein expression in stress-induced β-cells by using a molecular experiment method [16]. Thus, the molecular mechanisms of cinnamomi cortex effects will eventually be elucidated.

This is the first report that cinnamomi cortex stimulated PPARα-mediated transactivation. To clarify this mechanism, we performed a molecular experiment. In preliminary data, nuclear receptor coactivator SRC-1 [17] binding to PPARα was not enhanced by adding cinnamomi cortex in a GST pull-down assay. These findings suggested that activating PPARα by cinnamomi cortex would not be mediated through protein-protein interaction between PPARα and its coactivators.

In our preliminary data, other herbal medicines such as Shoseiryuto (2.3-fold) and Keishikajutubuto (2.1-fold), including cinnamomi cortex as components, also activated PPARα-mediated transactivation in CV1 cells under the same conditions. As herbal medicine is not simply a purified substrate but contains many ingredients, the activating degree may be different in each.

Hachimi-jio-gan was reported to prevent diabetic kidney damage by reducing renal oxidative stress or attenuating glucose toxicity [18]. In addition, PPARα is highly expressed in the kidney [19]; however, the function of PPARα in the kidney has not been identified. Recently, the role of PPARα in the kidney has been analyzed in several experiments. PPARα-deficient mice showed severe diabetic nephropathy through an increase in extracellular matrix formation and inflammation [20]. Moreover, a PPARα agonist, fenofibrate improved glomerular hypertrophy and mesangial matrix expansion, resulting in reduced urinary albumin excretion in db/db mice [21]. Combining these data with our findings, one possibility is that improvement by Hachimi-jio-gan of renal disease may be mediated via PPARα stimulation. Although the reason why PPARα-mediated transactivation was specifically activated by cinnamomi cortex in renal cells is not fully explained at present, our findings may lead to the development of novel therapy for the prevention of diabetic nephropathy through PPARα activation, not only by Hachimi-jio-gan but also by cinnamomi cortex.

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References

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