—Regular Articles—

Pharmacological Effects of Hachi-mi-jio-gan Extract (Harncare®) on the Contractile Response and on Pharmacologically Relevant Receptors in the Rat Bladder

Yoshihiko ITO,[a] Masanao SEKI,[a] Yasuhiko NISHIOKA,[b] Masatoshi KIMURA,[b] Akio YASUDA,[b] Tsukasa KIRIMOTO,[b] Toshikazu YASUDA,[c] and Shizuo YAMADA*,[a]

[a]*Department of Pharmacokinetics and Pharmacodynamics and Global COE Program, School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan,* [b]*Consumer Healthcare Product Strategy Planning, Taiho Pharmaceutical Co. Ltd., 224–2, Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771–0194, Japan, and* [c]*Hashima Laboratory, Nihon Bioresearch Inc., 104, 6-chome, Majima, Fukuju-cho, Hashima, Gifu 501–6250, Japan*

(Received February 19, 2009; Accepted April 30, 2009; Published online May 14, 2009)

Hachi-mi-jio-gan extract (Harncare®; HE), a galenical produced from the traditional Chinese herbal mixture Ba-Wei-Die-Huang-Wan, has been reported to improve lower urinary tract symptoms (LUTS) in patients. The present study was undertaken to clarify the pharmacological effects of HE on smooth muscle contraction and on pharmacologically relevant (muscarinic, 1,4-DHP and purinergic) receptors in the rat bladder. Additionally, the effects of repeated oral treatment with HE on the hepatic cytochrome P-450 (CYP) and on blood biochemical values in rats were examined. HE (10 mg/ml) inhibited significantly the acetylcholine-induced contraction of isolated rat bladder strips. The pD_2 value in the absence and presence of HE (10 mg/ml) was 5.14 ± 0.16 and 3.99 ± 0.17, respectively. HE (0.1 to 10 mg/ml) inhibited the specific binding of [*N*-methyl-^{3}H] scopolamine methyl chloride ([^{3}H]NMS), (+)-[^{3}H]PN 200–110 and $\alpha\beta$-methylene ATP [2,8-^{3}H] tetrasodium salt ([^{3}H]$\alpha\beta$-MeATP) in the rat bladder in a concentration-dependent manner. The respective IC_{50} values were 6.85 ± 0.94, 7.08 ± 0.72 and 1.34 ± 0.23 mg/ml. Based on IC_{50} values, the binding activity of HE for purinergic receptors was shown to be significantly (about 7 times) greater than that for muscarinic and 1,4-DHP receptors. Repeated oral administration of HE (10, 30 and 100 mg/kg/day) for 4 weeks had little or no effect on the level and activity of hepatic CYP or on blood biochemical values in rats. In conclusion, the present study has shown that HE exerts significant binding activity for pharmacologically relevant receptors in the rat bladder and a relaxant effect on the acetylcholine-induced contraction of isolated muscle strips. HE seemed to exhibit little pharmacokinetic interaction with drugs.

Key words——Hachi-mi-jio-gan; lower urinary tracts symptom; bladder; contraction; pharmacologically relevant receptor

INTRODUCTION

The number of patients with lower urinary tract symptoms (LUTS) such as urinary incontinence and urinary frequency is increasing, especially among the elderly.[1] Anticholinergic drugs are the first choice in the treatment of LUTS. These agents inhibit the cholinergic contraction of bladder smooth muscle by blocking muscarinic receptors.

Hachi-mi-jio-gan (Ba-Wei-Di-Huang-Wan) is a traditional Chinese herbal preparation, composed of eight natural ingredients; Rehmanniae radix, Corni fructus, Dioscoreae rhizoma, Alismatis rhizoma, Hoelen, Moutan cortex, Cinnamomi cortex and Aconiti tuber. It has been used for the treatment of diabetes mellitus, hypertension, nephrotic syndromes, and glomerulonephritis since the late middle ages.[2,3] In Japan, Hachi-mi-jio-gan extract (Harncare®: HE) is currently used for the treatment of LUTS.[4] However, the effects of HE on bladder smooth muscle tone and autonomic receptors in the lower urinary tract have not been investigated.

Herbal products, including HE, are used frequently with other prescription medications. Thus, the potential for serious pharmacokinetic and pharmacodynamic interactions with drugs, particularly in elderly patients who are susceptible to the consequences of drug interactions, should be a concern.[5] There is little direct information about the pharmacokinetic effects of HE on hepatic drug-metabolizing (cyto-

*e-mail: yamada@u-shizuoka-ken.ac.jp

chrome p450: CYP) enzymes. Thus, the aim of this study was to clarify the effects of HE on the contraction of bladder smooth muscle, pharmacologically relevant receptors in the bladder, hepatic CYP and blood biochemical values in rats.

MATERIALS AND METHODS

Materials HE was produced by Taiho Pharmaceutical Co. Ltd., Tokyo, Japan. The composition of the plant mixture used to obtain Harncare is given in Table 1; this mixture was extracted with boiling water and the ethanol was added. The decoction was centrifuged to obtain the aqueous extract. [*N*-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS, 2.979 TBq/mmol), (+)-[^{3}H]PN 200–110 (3.180 TBq/mmol), and [^{3}H]$\alpha\beta$-methylene adenosine triphosphate [2,8-^{3}H]tetrasodium salt ([^{3}H]$\alpha\beta$-MeATP, 580.9 GBq/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). Resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, 6β-hydroxyltestosterone, corticosterone, testosterone, ketoconazole, *p*-nitrophenol, 4-nitrocatechol and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO). (*S*)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). All other chemicals were purchased from commercial sources.

Analysis of 3D-HPLC Fingerprints of HE HE (0.5 ml) was diluted with methanol (20 ml). The solution was filtrated (0.45 μm) and then subjected to HPLC. The HPLC system comprised a pump (LC-10A; Shimadzu, Kyoto, Japan), a TSK-GEL, ODS-80TS column (ϕ4.6$\times$250 mm, Tosoh, Japan), and solvents (A) 0.05 M AcOH-AcONH$_4$ and (B) CH_3CN. A linear gradient of, by volume, 90% A and 10% B changing over 60 min to 0% A and 100% B was used. The flow rate was controlled with the LC-10A at 1.0 ml/min. The eluate from the column was monitored, and the three-dimensional data were processed with a diode array detector (SPD-M10A; Shimadzu, Kyoto, Japan). The three-dimensional HPLC charts of the methanol solutions of HE are shown in Fig. 1. Loganin and paeoniflorin were detected as the major compounds of HE, while benzoylmesaconine, cinnamic acid, cinnamaldehyde and paeonol were also observed.

Table 1. Crude Drug Composition of Hachi-mi-jio-gan Extract (Harncare®; HE)

Plant name	Composition (g)
Rehmanniae Radix (*Rehmannia glutinosa* LIBOSCH)	5.0
Corni Fructus (*Cornus officinalis* SIEB *et* ZUCC)	3.0
Dioscoreae Rhizoma (*Dioscorea japonica* THUNB)	3.0
Alismatis Rhizoma (*Alisma orientale* JUZEP)	3.0
Poria (*Poria cocos* WOLF)	3.0
Moutan Cortex (*Paeonia suffruticosa* ANDREWS)	3.0
Cinnamomi Cortex (*Cinnamomum cassia* BLUME)	1.0
Processi aconiti Radix (*Aconitum carmichaeli* DEBX)	1.0

As provided by Taiho Pharmaceutical Co. Ltd., Tokyo, Japan.

Animals and Administration of HE Male Sprague-Dawley rats weighing 250 to 350 g were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Rats were housed with a 12-hour light-dark cycle and fed laboratory food and water *ad libitum*. In the drug-metabolizing enzyme analysis and blood biochemical examination, rats received orally HE (10, 30 and 100 mg/kg/day) for 4 weeks. The control animals received vehicle alone. This study was done according to guidelines approved by the experimental animal ethics committee of the University of Shizuoka.

Acetylcholine-induced Contraction in Bladder Smooth Muscle Strips Urinary bladder strips were isolated from the rats. The abdomen was opened, and the urinary bladder was dissected. Urinary bladder strips were cut longitudinally into two or three strips. The bladder muscle strips were suspended in a tissue bath filled with Krebs-Henseleit solution. The strips were maintained at 37°C in an atmosphere of 95% O_2 and 5% CO_2. The response of the strips was measured isometrically under a resting tension of 1 g using a transducer (TB-612T, Nihon Kohden, Tokyo, Japan).

The concentration-response curves for acetylcholine were obtained by increasing the concentration in a stepwise manner after the response to the previous concentration had reached a plateau. The cumulative concentration-response curves were reproducible by applying acetylcholine at several times by appropriate intervals. The strips were then incubated with HE for 5 min and the concentration-response curves for acetylcholine were obtained in the presence of in-

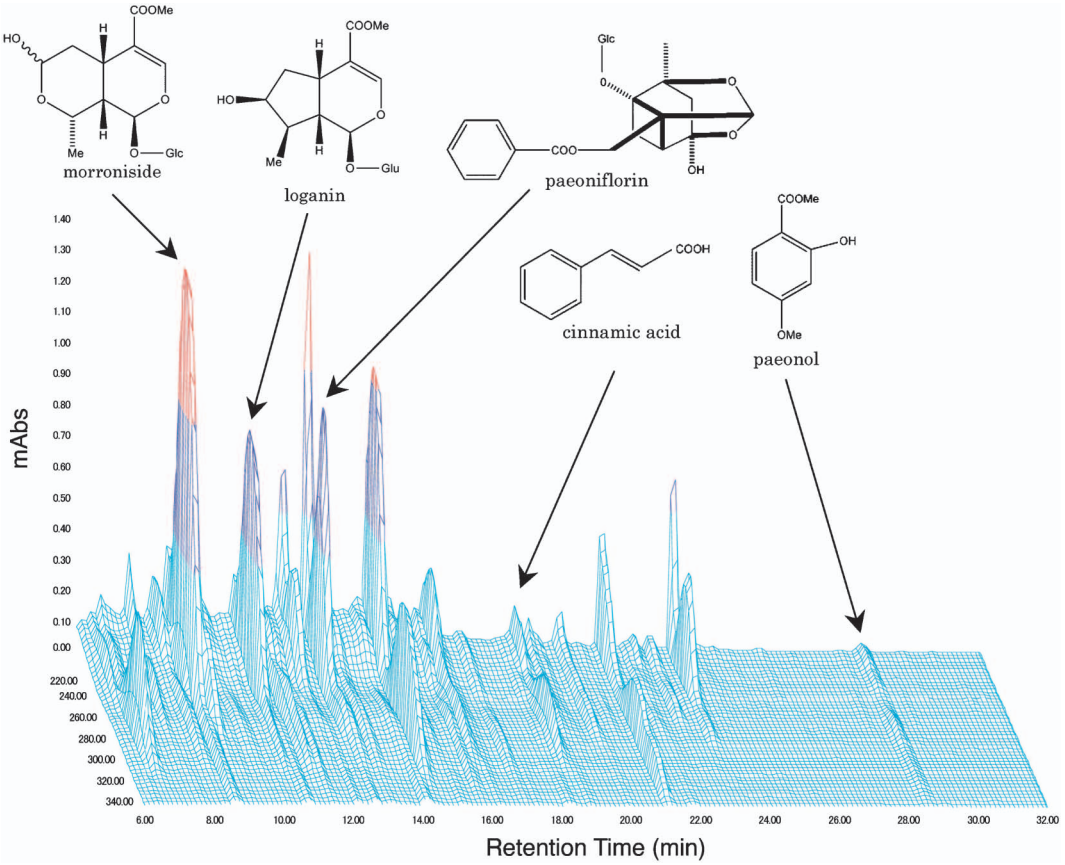

Fig. 1. Three-dimensional HPLC Profile of the Methanol Solution of Hachi-mi-jio-gan Extract (Harncare®)

creasingly higher concentrations of HE.

Binding Assays for [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H]$\alpha\beta$-MeATP The radioligand-binding assays for muscarinic receptors, 1,4-DHP receptors and purinergic receptors were performed using [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H]$\alpha\beta$-MeATP respectively, as previously described[6]. Rats were exsanguinated by taking the blood from the descending aorta under temporary anesthesia with diethyl ether and the aorta. The bladder and submaxillary gland were then dissected and the tissues were minced with scissors. The submaxillary gland was utilized to examine the binding activity of HE in exocrine muscarinic receptors. The difference between the bladder and submaximally gland in the muscarinic binding activity of HE may be interesting issue because antimuscarinic agents for the treatment of LUTS cause frequently dry mouth as one of systemic side effects. In the case of binding assays for [^{3}H]NMS and (+)-[^{3}H]PN 200–110, the tissues were homogenized using a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland) in 19 volumes of ice-cold 30 mM Na^+/HEPES buffer (pH 7.5). The homogenates were then centrifuged at 40000×gravity for 20 min at 4°C. The resulting pellets were finally resuspended in the ice-cold buffer. For the [^{3}H]$\alpha\beta$-MeATP binding assay, the bladder was homogenized in 10 volumes of ice-cold 50 mM Tris /HCl buffer (pH 7.5) containing 1 mM EDTA, 2 μg/ml soybean trypsin inhibitor, and 10 μg/ml bacitracin. The homogenate was then centrifuged at 2000×gravity for 10 minutes at 4°C. The resulting pellet suspension was further centrifuged at 2000×gravity for 10 minutes at 4°C, and the supernatant was combined with the original supernatant. The supernatant was centrifuged at 48000×gravity for 20 minutes at 4°C and the suspension of the resulting pellet was used. In the displacement experiments, the rat tissue homogenates were incubated with [^{3}H]NMS (0.46 nM), (+)-[^{3}H]PN 200–110 (0.50 nM) and [^{3}H]$\alpha\beta$-MeATP (4.5 nM) in the presence of various concentrations (0.1 to 100 mg/ml) of HE. Incubation was carried out for 60 min ([^{3}H]NMS and (+)-[^{3}H]PN 200–110) and 120 min ([^{3}H]$\alpha\beta$-MeATP) at 25°C (in

the dark with a sodium lamp in the case of (+)-[^{3}H]PN 200–110). The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters and the filters were rinsed with ice-cold buffer. Tissue-bound radioactivity was extracted from the filters by immersion in scintillation fluid, and radioactivity was determined by a liquid scintillation counter. Specific binding of [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H]$\alpha\beta$M-MeATP was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine, 1 μM nifedipine and 3 μM $\alpha\beta$-MeATP, respectively.

Preparation of Hepatic Microsomal and Cytosolic Fractions After repeated oral administration of HE (10, 30, 100 mg/kg/day) in rats, the liver was dissected and stored at −80°C prior to analysis. The liver was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 10000×gravity for 30 min at 4°C. The supernatant was further centrifuged at 105000×gravity for 60 min at 4°C. The supernatant was used as the cytosolic fraction for the assay of glutathione S-transferase, the activity of which was determined by the method of Habig *et al.*[7] using 1-chloro-2,4-dinitrobenzene as a substrate. The pellet was washed once with 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose by centrifugation at 105000×gravity for 60 min at 4°C, and the concentration and activities of CYP were analyzed. Protein concentrations of microsomal and cytosolic fractions were determined using a BCA protein assay kit (Pierce, Rockford, IL).

Analysis of Drug Metabolizing Enzymes The CYP content was quantified by the method of Omura *et al.*[8] The activities of glutathione S-transferase and various CYP enzymes were determined by HPLC as reported previously.[9] The subtypes of drug-metabolizing enzymes (corresponding CYPs) were methoxyresorufin O-demethylase (CYP1A2), ethoxyresorufin O-deethylase (CYP1A1), pentoxyresorufin O-dealkylase (CYP2B), p-nitrophenol hydroxylase (CYP2E1), testosterone 6β-hydroxylase (CYP3A) and (S)-warfarin 7-hydroxylase (CYP2C9).

Blood Biochemical Examination After repeated oral administration of HE (10, 30, 100 mg/kg/day), the concentrations of total protein, albumin, triglyceride, phospholipids, total cholesterol, AST, ALT, ALP and γ-GTP in plasma of rats were determined by Clinical Analyzer 7170 (HITACHI).

Data Analysis For the analysis of acetylcholine-induced contractions of the isolated rat bladder, pD_2 values were calculated as described.[10] The value was defined as the negative logarithm of the concentration of acetylcholine which evokes 50% of its maximal effect. Analyses of binding data for [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H] $\alpha\beta$-MeATP were performed as detailed previously.[11] The binding activities of SPE for muscarinic cholinoceptors and 1,4-DHP calcium channel antagonist receptors were estimated based on IC_{50} values, which are the concentrations of HE necessary to displace 50% of specific binding of [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H] $\alpha\beta$-MeATP (determined by a log probit analysis). Statistical analyses of the data were performed by a one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons, and a value of $p<0.05$ was considered significant.

RESULTS

Relaxant Effect on the Isolated Rat Bladder Figure 2 shows the concentration-response curves for acetylcholine-induced contractions of the isolated rat bladder strips in the absence and presence of HE (1, 10 mg/ml). HE at a concentration of 1 mg/ml had little effect on the acetylcyholine-induced contraction of the bladder, but at 10 mg/ml significantly inhibited the response. The pD_2 value for the control, and 1 and 10 mg/mL of HE was 5.14±0.16, 5.17±0.18 and 3.99±0.17, respectively. The pD_2 value at 10

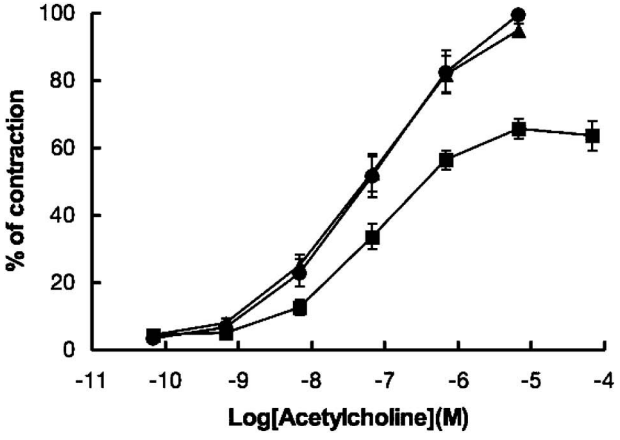

Fig. 2. Effect of Hachi-mi-jio-gan Extract (Harncare®; HE) on the Concentration-response Curves for the Acetylcholine-induced Contraction of the Isolated Rat Bladder Strips

The contractile responses of the bladder strips to acetylcholine (68.4 pM to 68.4 μM) were measured in the absence (control, ●) and presence of HE (1 mg/ml, ▲; 10 mg/ml, ■). Each point represents the mean±S.E. for 5 separate experiments.

mg/ml of HE was significantly ($p<0.05$) lower than the control value. In contrast, HE (1, 10 mg/ml) had little inhibitory effect on KCl (100 mM)-induced contraction in rat bladder muscles (data not shown).

Effect on the Pharmacologically Relevant Receptors in the Bladder HE (0.1 to 10 mg/ml) inhibited the specific binding of [^{3}H]NMS in the bladder and submaxillary gland of rats in a concentration-dependent manner (Fig. 3A), with IC_{50} values of 6.85 ± 0.94 and 13.1 ± 0.8 mg/ml, respectively. The IC_{50} value was significantly ($p<0.05$) lower in the bladder than in the submaxillary gland. Similarly, HE (0.1 to 10 mg/ml) inhibited the specific binding of [^{3}H] $\alpha\beta$-MeATP (Fig. 3B) and (+)-[^{3}H]PN 200–110 (Fig. 3C) in the bladder, in a concentration-dependent manner: IC_{50} values were 1.34 ± 0.23 and 7.08 ± 0.72 mg/ml, respectively. The IC_{50} value for [^{3}H] $\alpha\beta$-MeATP was significantly ($p<0.01$) lower than the values for [^{3}H] NMS and (+)-[^{3}H]PN 200–110.

Effects of Repeated Oral Administration on Hepatic Drua-Metabolizing Enzymes and Blood Biochemical Values in Rats Repeated oral administration of HE (10, 30 100 mg/kg/day) had little significant effect on body weight, CYP content and the activities of drug-metabolizing enzymes (glutathione S-transferase, ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, pentoxyresorufin O-dealkylase, *p*-nitrophenol hydroxylase, testosterone 6β-hydroxylase and (*S*)-warfarin 7-hydorylase) in the rat liver, compared with the corresponding control values (Table 2). Similarly, the repeated administration of HE had little effect on total protein, albumin, triglyceride, phospholipid, total cholesterol, GOT, GPT, ALP and γ-GTP levels in rat blood (Table 3).

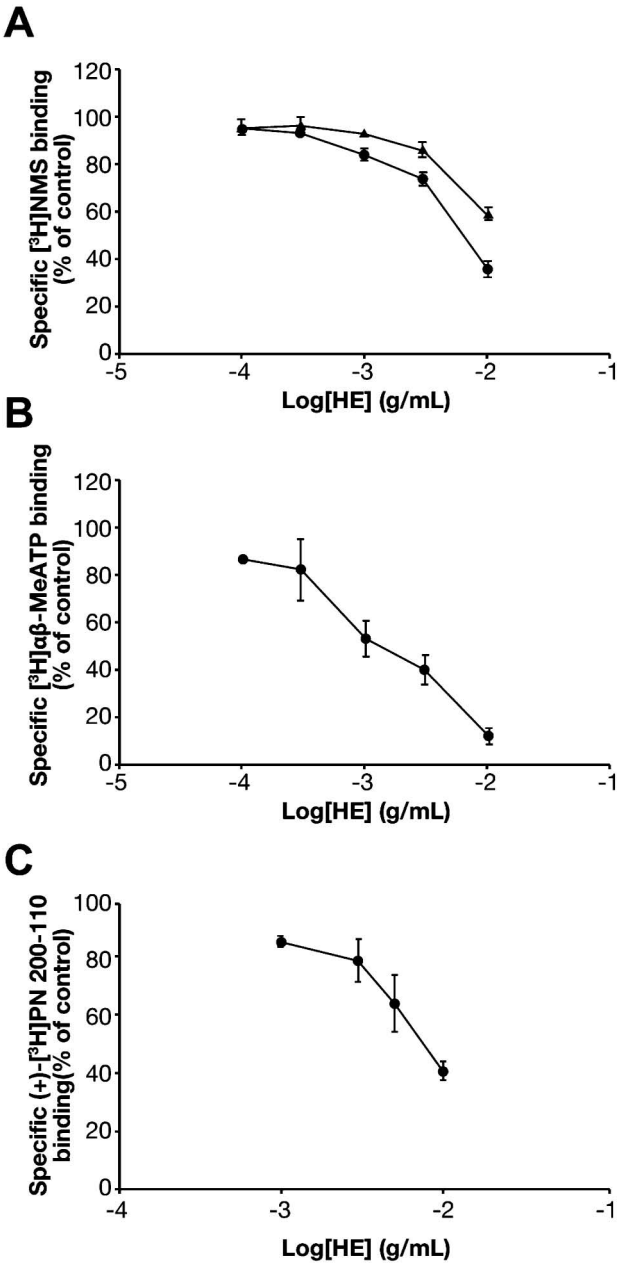

Fig. 3. Effects of Hachi-mi-jio-gan Extract (Harncare®; HE) on the Specific Binding of [^{3}H]NMS (A), [^{3}H]$\alpha\beta$-MeATP (B) and (+)-[^{3}H]PN200–110 (C) in the Rat Bladder (●) and Submaxillary Gland (▲)

The specific binding of [^{3}H]NMS (0.46 nM), [^{3}H] $\alpha\beta$-MeATP (4.5 nM) and (+)-[^{3}H]PN200–110 (0.50 nM) in homogenates of rat bladder and submaxillary gland was measured in the absence and presence of 4 or 5 concentrations (100 μg/ml to 10 mg/ml) of HE. Each point represent the mean±S.E. for 5 separate experiments.

DISCUSSION

The usage of medicinal herbs has grown fast as complementary and alternative medicine,[12] but scientific knowledge of the efficacy and safety of herbs is still lacking. Furthermore, the potential for interactions between herbs and drugs should be a concern because all herbs contain a large number of constituents.

HE is an extract of Hachi-Mi-Ji-O-Gan obtained using ethanol. HE is used clinically for the treatment of LUTS.[13,14] Hachi-Mi-Ji-O-Gan has been shown to improve significantly maximum flow rates and to reduce significantly scores of emptying, weak stream, total scores and quality of life scores according to the International Prostate Symptom Score (IPSS).[13] In a canine study using cystometry, Hachi-Mi-Ji-O-Gan inhibited the frequency of rhythmic bladder contractions and significantly increased both maximum vesical volume and effective vesical volume.[14]

Table 2. CYP Contents and Activities of Hepatic Drug-Metabolizing Enzymes in Rats Orally Administered Hachi-mi-jio-gan Extract (Harncare®; HE) at Doses of 1, 30 and 100 mg/kg for 4 Weeks

	Control	10 mg/kg	30 mg/kg	100 mg/kg
Hepatic drug-metabolizing enzymes				
Cytochrome P450 (CYP) content (nmol/mg protein)	0.83±0.04	0.84±0.05	0.81±0.01	0.76±0.08
Glutathione S-transferase activity (nmol/mg protein/min)	0.84±0.07	0.77±0.04	0.82±0.05	0.80±0.05
Ethoxyresorufin O-deethylase (CYP1A1) activity (pmol/mg protein/min)	42.2±4.9	39.5±2.5	44.0±4.6	45.5±3.9
Methoxyresorufin O-demethylase (CYP1A2) activity (pmol/mg protein/min)	29.0±1.8	29.6±2.4	31.5±3.2	31.0±1.8
Pentoxyresorufin O-dealkylase (CYP2B) activity (pmol/mg protein/min)	17.3±3.9	13.1±2.9	11.4±1.6	16.7±6.0
p-Nitrophenol hydroxylase (CYP2E1) activity (pmol/mg protein/min)	16180±1644	15728±1754	17372±2290	16440±1414
Testosterone 6β-hydroxylase (CYP3A) activity (pmol/mg protein/min)	2533±325	2651±407	2410±350	2549±197
(*S*)-Warfarin 7-hydroxylase (CYP2C9) activity (pmol/mg protein/min)	2.21±0.30	2.08±0.19	2.30±0.26	2.43±0.27

Values are the mean±S.E. for 5 to 7 rats.

Table 3. Biochemical Parameters in Blood of Rats Orally Administered Hachi-mi-jio-gan Extract (Harncare®; HE) at Doses of 1, 30 and 100 mg/kg for 4 Weeks

	Control	10 mg/kg	30 mg/kg	100 mg/kg
Total protein (g/dl)	6.4±0.1	5.7±0.5	5.9±0.3	3.7±1.5
Albumin (g/dl)	4.4±0.1	4.2±0.2	4.1±0.2	2.6±1.3
Triglyceride (mg/dl)	51.4±4.2	59.5±11.3	48.0±15.6	34.9±5.0
Phospholipid (mg/dl)	94.4±1.3	99.7±9.2	92.8±8.5	59.5±6.2
Total cholesterol (mg/dl)	54.6±1.1	58.7±7.2	58.0±6.0	35.9±4.7
GOT (IU/l at 37°C)	94.6±9.1	88.7±4.4	81.0±4.6	55.5±6.2
GPT (IU/l at 37°C)	37.6±2.0	39.3±3.1	34.0±2.2	23.2±4.0
ALP (IU/l at 37°C)	718.4±41.8	592.0±65.0	610.2±57.1	405.5±16.4
γ-GTP (IU/l at 37°C)	<2	<2	<2	<2

GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, ALP: alkalinephosphatase, γ-GTP: γ-glutamyl transpeptidase. Values are mean±S.E. for 5 to 7 rats.

There are many potential targets in the treatment of LUTS because its pathophysiology is multifactorial.[15] Pharmacologically relevant receptors such as muscarinic receptors, 1,4-DHP receptors and purinergic receptors in the bladder are considered as targets of therapeutic agents for LUTS. The present study has shown that HE inhibited the acetylcholine-induced contractions of rat bladder as well as the specific binding of [^{3}H]NMS, (+)-[^{3}H]PN 200–110 and [^{3}H] $\alpha\beta$-MeATP in the bladder of rats, in a concentration-dependent manner. These results indicate that HE may bind to muscarinic receptors, 1,4-DHP receptors and purinergic receptors in the bladder. Thus, the inhibitory effects of HE on the acetylcholine-induced contractions of bladder muscle strips are mediated at least in part *via* antagonistic effects on muscarinic cholinoceptors. Since HE exerted little inhibitory effect on the KCl-induced contraction of isolated rat bladder strips, it is unlikely that HE inhibits directly the contractile elements of bladder smooth muscles. Furthermore, HE displayed approximately 2-fold lower binding affinity to the muscarinic receptors in the submaxillary gland than in the bladder (Fig. 3A).

Notably, based on IC_{50} values, the binding activity of HE for purinergic receptors in the rat bladder was shown to be significantly (about 7 times) greater than that for muscarinic and 1,4-DHP receptors. Thus, it is likely that HE exerts a significantly greater effect on the functions of purinergic receptors in the bladder. The binding activity of HE for purinergic receptors in the bladder is consistent with the recent finding that HE treatment reduced the expression of purinergic

$P2X_3$ receptors and inhibited ATP-induced detrusor overactivity in the bladder.[16] Furthermore, the current finding provides pharmacological evidence to support the notion that purinergic receptor-related mechanisms in the urothelium and smooth muscle may be altered in the bladder of patients with interstitial cystitis (IC).[17] ATP levels were significantly elevated in the urine of patients with IC and the stretch-activated release of ATP was augmented in the urothelium.[18] Rapp *et al.*[19] have suggested that purinergic (P2X) receptors may be a potential target for the treatment of disorders of the bladder. Thus, pharmacological agents that modify the functions of P2X receptors in the bladder are considered to counteract the symptoms mediated by abnormal purinergic signaling.[19] Taken together, HE might have some beneficial effects on patients with IC.

The potential for interactions between plant extracts and drugs should be a concern because the extracts contain a large number of constituents.[20–22] It is well established that St. John's wort (Hypericum perforatum) induces significantly hepatic CYP3A4 activity, thereby reducing the efficacy of therapeutic drugs such as cyclosporin, indinavir, and digoxin.[23] Repeated oral administration of HE had little significant influence on the levels and activities of hepatic drug-metabolizing enzymes (CYP isoforms) or on blood biochemical parameters in rats. Therefore, it is unlikely that HE alters the disposition of coadministered drugs.

In conclusion, HE has been shown to exert significant binding activity for pharmacologically relevant receptors in the rat bladder with the inhibition of acetylcholine-induced contractions of smooth muscle. These effects of HE may be partly relevant in alleviating the urination dysfunction in patients with LUTS. Consequently, the present study may provide further pharmacological evidence of the efficacy and safety of HE in patients with LUTS.

Acknowledgements This study was in part supported by Grants-in-Aid from Food Safety Commission, Japan (No. 0807).

REFERENCES

1) Milsom I., Abrams P., Cardozo L., Roberts R. G., Thuroff J., Wein A. J., *BJU Int.*, **87**, 760–766 (2001).
2) Hirotani Y., Ikeda T., Yamamoto K., Kurokawa N, *Biol. Pharm. Bull.*, **30**, 1015–1020 (2007).
3) Kiga C., Nakagawa T., Koizumi K., Sakurai H., Shibagaki Y., Ogawa K., Goto H., Saiki I., *Biol. Pharm. Bull.*, **28**, 1031–1037 (2005).
4) Kawahara K., Mori, K., *Voiding Disorders Digest*, **15**, 93–100 (2007).
5) Stewart, R. B., Cooper J. W., *Drugs Aging*, **4**, 449–461 (1994).
6) Takahashi D., Oyunzul L., Onoue S., Ito Y., Uchida S., Simsek R., Gunduz M. G., Safak C., Yamada S., *Biol. Pharm. Bull.*, **31**, 473–479 (2008).
7) Habig W. H., Jakoby, W. B., *Methods Enzymol.*, **77**, 398–405 (1981).
8) Omura T., Sato R., *J. Biol. Chem.*, **239**, 2370–2378 (1964).
9) Umegaki K., Saito K., Kubota Y., Sanada H., Yamada, K., Shinozuka K., *Jpn. J. Pharmacol.*, **90**, 345–351 (2002).
10) Ariens E. J., Van Rossum J. M., *Arch. Int. Pharmacodyn. Ther.*, **110**, 275–299 (1957).
11) Yamada S., Yamamura H. I., Roeske W. R., *J. Pharmacol. Exp. Ther.*, **215**, 176–185 (1980).
12) Eisenberg D. M., Davis R. B., Ettner S. L., Appel S., Wilkey S., Van Rompay M., Kessler R. C., *JAMA*, **280**, 1569–1575 (1998).
13) Yoshimura K., Terai A., Arai Y., *Hinyokika Kiyo*, **49**, 509–514 (2003).
14) Suzuki T., Okazaki H., Suzuki K., Kurokawa K., Higashi H., Suzuki K. *Kitakanto Med. J.*, **50**, 15–19 (2000).
15) Andersson K. E., *Neurourol. Urodyn.*, **26**, 934–947 (2007).
16) Imamura T., Ishizuka O., Zhong C., Ogawa T., Nakayama T., Kurizaki Y., Tanabe T., Nishizawa O., *Neurourol. Urodyn.*, published online DOI: 10.1002/nau.20702 (2009).
17) Kageyama A., Fujino T., Taki Y., Kato Y., Nozawa Y., Ito Y., Yamada S., *Neurosci. Lett.*, **436**, 81–84 (2008).
18) Sun Y., Keay S., De Deyne P. G., Chai T. C., *J. Urol.*, **166**, 1951–1956 (2001).
19) Rapp D. E., Lyon M. B., Bales G. T., Cook S. P., *Eur. Urol.*, **48**, 303–308 (2005).
20) Izzo A. A., Ernst E., *Drugs*, **61**, 2163–2175 (2001).
21) Williamson E. M., *Phytomedicine*, **8**, 401–409

(2001).
22) Sugiyama T., Kubota Y., Shinozuka K., Yamada S., Wu J., Umegaki K., *Life Sci.*, **75**, 1113–1122 (2004).
23) Roby C. A., Anderson G. D., Kantor E., Dryer D. A., Burstein A. H., *Clinical Pharmacol. Ther.*, **67**, 451–457 (2000).