

Full Paper

Mechanisms Underlying Mechanical Responses to *Ephedra herb* of Isolated Rabbit Urinary Bladder and Urethra, a Possible Stress Urinary Incontinence TherapeuticKazuhide Ayajiki¹, Toshio Kimura², Kohei Yamamizu¹, and Tomio Okamura^{1,*}¹Department of Pharmacology, Shiga University of Medical Science, Seta, Otsu 520-2192, Japan²Department of Obstetrics and Gynecology, Yasu Hospital, Koshinohara 1094, Yasu 520-2331, Japan

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Abstract. To compare the mechanisms underlying mechanical responses to ephedrine and *Ephedra herb*, a main component of *Kakkon-to*, in isolated male and female rabbit urinary bladder and urethral strips, responses of isolated strips to the agents were recorded in organ bath systems. Ephedrine and *Ephedra herb* relaxed the female urinary bladder to the similar extent. These relaxations are reversed to contractions by timolol. In the presence of timolol, ephedrine produced less contraction of urethral strips in the female than those in the male; this contraction was abolished by prazosin. *Ephedra herb* contracted the female urethra less than that of the male, and the contraction was stronger than that by ephedrine. The contraction caused by *Ephedra herb* in strips treated with timolol was significantly inhibited by prazosin. The prazosin-resistant contraction of the female urethra was greater than that of the male. Quinacrine, a phospholipase A₂ inhibitor, indomethacin, and AA861, a 5-lipoxygenase inhibitor, inhibited the contraction. The contraction was inhibited by ZK 158252, a leukotriene (LT) B₄-receptor antagonist. These findings suggest that *Ephedra herb* contracts the urethra via arachidonic acid metabolites together with α_1 -adrenoceptor stimulation. The metabolites produced by 5-lipoxygenase may stimulate LTB₄, but not CysLT₁, receptors. These contractile components induced by *Ephedra herb* and *Kakkon-to* might be effective for the treatment of stress urinary incontinence.

Keywords: stress urinary incontinence, *Ephedra herb*, ephedrine, urethral contraction, arachidonic acid metabolite

Introduction

Women have higher rates of urinary incontinence than men, which increase with age; one third of women older than 65 years of age have some degree of incontinence (1). Urinary incontinence is roughly classified into urge and stress incontinence. The combination of non-medical therapy including biofeedback-assisted behavioral training and medication of anticholinergic agents, oxybutynin and tolterodine, for urge incontinence results in a better control of the incontinence than either treatment alone (2). On the other hand, when treating female patients with stress urinary incontinence (SUI), physicians should

consider various therapies including pelvic floor muscle exercises, intravaginal support devices, pessaries, urethral occlusion inserts, medication, and surgery (3). As one of the medications, estrogens have been used for the treatment of urinary incontinence since as early as 1941 (4). However, in a recent analysis of the medication, little benefit of estrogens for SUI was found (5). Furthermore, α -adrenoceptor agonists have been found to be effective for SUI in clinical trials, but the agonists lack exclusive selectivity for urethral α -adrenoceptors and may cause elevation of blood pressure, palpitation, sleep disturbance, and headache (6). Thus, the Food and Drug Administration approves no drugs for SUI at the present time (3).

Kakkon-to, a traditional Chinese herbal medicine, has been used for the treatment of common cold, tonsillitis, and chronic inflammatory diseases (7).

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Recently, Japanese gynecologists noticed on the basis of their experiences that *Kakkon-to* is effective for SUI because it decreases the scores of the international consultation on incontinence questionnaire short form composed of 3 categories: frequency, severity, and quality-of-life impact of urinary incontinence (8). However, mechanisms of actions on SUI of *Kakkon-to* are unknown.

Ephedra herb is a major component of *Kakkon-to*. Ephedrine extracted from *Ephedra herb* is classified as a miscellaneous adrenergic agonist (9). Ephedrine was found to be effective for SUI in clinical trials (10).

Therefore, in the present study, we compared the mechanisms underlying mechanical responses of isolated urinary bladder and urethra to ephedrine and *Ephedra herb* in male and female Japanese White rabbits in order to explain a possible mechanism by which *Kakkon-to* clinically works as a therapeutic for female SUI.

Materials and Methods

Animals

Eight male and twenty female Japanese White rabbits, weighing 3.0–4.0 kg, were used for the present study. The Animal Care and Use Committee at Shiga University of Medical Science approved the use of rabbit urinary bladder and urethra in this study.

Mechanical response

Under deep general anesthesia with ketamine (20 mg/kg, i.m.) and sodium pentobarbital (25 mg/kg, i.v.), Japanese White rabbits were killed by bleeding from common carotid arteries. The urinary bladder was isolated, and the largest part of the corpus of the bladder was transversely cut into several open-ring strips of approximately 20-mm length. The urethra close to the ostium urethrae was isolated and cut into an open-ring strip of approximately 20-mm length. The strips were fixed vertically between hooks in an organ chamber containing a modified Ringer-Locke solution, as previously reported (11). The resting tension was adjusted to 1.5 g.

Isometric contractions and relaxations were displayed on an ink-writing recorder. The contractile response to 5 mM Ba^{2+} was obtained first, and the urinary bladder and urethral strips were repeatedly washed with fresh media and equilibrated. The strips of urinary bladder were partially contracted with prostaglandin (PG) $\text{F}_{2\alpha}$ ($0.2 - 1 \times 10^{-6}$ M), the contraction being in the range between 35%–40% of the contraction induced by 5 mM Ba^{2+} . Concentration–response curves for ephedrine and *Ephedra herb* were obtained by adding the drug directly to the bathing media in cumulative concentrations. The

initial responses to the drugs were compared in the absence or presence of timolol. At the end of each series of the experiments, papaverine (10^{-4} M) was applied to attain the maximum relaxation. Relaxations induced by ephedrine and *Ephedra herb* in the urinary bladder strips are expressed relative to those induced by papaverine as 100% and contractions induced by the drugs after treatment with β -blocker in the urinary bladder are expressed relative to those induced by 5 mM Ba^{2+} as 100%. In the case to compare the urethral responses to ephedrine and *Ephedra herb* between males and females, the contractions induced by the drugs are expressed relative to those induced by 5 mM Ba^{2+} . After reproducibility of the contractions caused by ephedrine and *Ephedra herb* under treatment with prazosin and timolol was determined in the case of urethra, the strips were treated with blocking agents. In order to evaluate these blocking agents contractions induced by *Ephedra herb* are expressed relative to the maximal contraction induced by 300 $\mu\text{g}/\text{ml}$ of *Ephedra herb* in the absence of blocking agents.

Drugs and statistics

The results shown in the text and figures are expressed as mean values \pm S.E.M. Statistical analyses were made using Student's unpaired *t*-test for two groups. Drugs used were $\text{PGF}_{2\alpha}$ (Pfizer Inc., Tokyo); ephedrine hydrochloride (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya); *Ephedra herb* (an extract of *Ephedra sinica*, Lot No. 2991037010; Tsumura & Co., Tokyo); prazosin hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka); atropine sulfate (Tanabe Seiyaku Co., Ltd., Osaka); yohimbine hydrochloride (Nacalai Tesque Inc., Kyoto); methysergide hydrogen maleate (Novartis AG, Basel, Switzerland); *d*-chlorphenylamine maleate (Schering-Plough Co., NJ, USA); AA861 (Takeda Pharmaceutical Co., Ltd., Osaka); montelukast (Merck & Co., Inc., NJ, USA); ZK 15252 (Bayer HealthCare, Berlin, Germany); timolol maleate, quinacrine dihydrochloride, and indomethacin (Sigma Chemical, St. Louis, MO, USA); and papaverine hydrochloride (Dainippon, Osaka). The powder of *Ephedra herb* was sufficiently mixed with distilled water, and the solution was filtrated with filter paper (Tokyo Roshi Kaisha, Ltd., Tokyo). Thereafter, the filtrate was diluted with distilled water in order to adjust the concentration to 10 mg/ml. ZK 15252 was dissolved in the solvent (4 ml of 99.5% ethanol + 0.05 ml of 1 M NaOH + 36 ml of distilled water).

Results

Response of urinary bladder strips from the female rabbits to ephedrine and *Ephedra herb*

Ephedrine and *Ephedra herb* relaxed the urinary bladders of female rabbits in a concentration-dependent manner. The maximal relaxations caused by these drugs were almost the same, and they were reversed to a slight contraction by treatment with 10^{-6} M timolol, a β -blocker (Fig. 1).

Response of urethral strips from male and female rabbits to ephedrine and *Ephedra herb*

Ephedrine contracted urethral strips of male and female rabbits in a concentration-dependent manner, and the magnitude of the contractions was not significantly different between male and female rabbits (Fig. 2, top left). Under treatment with 10^{-6} M timolol, contractile responses to ephedrine of the female urethra were significantly less than those of the male (Fig. 2, top center). The ephedrine-induced contractions in the male and female rabbits were abolished by additional treatment with 10^{-5} M prazosin, an α_1 -adrenoceptor blocker (Fig. 2, top right).

Ephedra herb contracted the urethral strips both of male and female rabbits in a concentration-dependent manner, and the magnitude of contractions in the female rabbits was significantly less than those in the male with and without 10^{-6} M timolol (Fig. 2, bottom left and center). Under treatment with 10^{-6} M timolol, the contractile responses to *Ephedra herb* in the male and female rabbits were not abolished by 10^{-5} M prazosin, and the magnitude of the contractions at $30 \mu\text{g/ml}$ of *Ephedra herb* in the female was slightly but significantly greater than that in the male ($10.8 \pm 4.1\%$ ($n = 5$) in the female and $1.2 \pm 0.5\%$ ($n = 5$) in the male; Fig. 2, bottom right).

Response of urethral strips from female rabbits to *Ephedra herb* in the presence of timolol and prazosin

Under combined treatment with 10^{-6} M timolol and 10^{-5} M prazosin, contractile responses to *Ephedra herb* in concentrations from $3 - 300 \mu\text{g/ml}$ of the female urethral strips were not significantly affected by treatment with 10^{-7} M atropine, a muscarine-receptor antagonist (12); 10^{-7} M yohimbine, an α_2 -adrenoceptor blocker (13); 10^{-6} M methysergide, a $5\text{-HT}_1 + 5\text{-HT}_2$ -receptor blocker (14); or 10^{-6} M chlorphenylamine, a H_1 -receptor blocker (15) (data not shown). The contractions were significantly inhibited by 3×10^{-5} M quinacrine, a phospholipase A_2 inhibitor (16); 10^{-6} M indomethacin, a cyclooxygenase inhibitor; and 10^{-5} M AA861, a 5-lipoxygenase inhibitor (17) (Fig. 3). Furthermore, contractile responses to *Ephedra herb* in the presence of timolol and prazosin were significantly inhibited by 10^{-4} M ZK 158252, but were not affected by 10^{-6} M montelukast (Fig. 4). The concentrations of ZK 158252 and montelukast used were reportedly sufficient to inhibit the receptor functions of LTB_4 (18) and CysLt_1 (19), respectively.

Discussion

The isolated urinary bladder strips from female rabbits were relaxed by ephedrine and *Ephedra herb* almost to the same extent. The relaxations were reversed to slight contractions by timolol. These findings indicate that both drugs relax the rabbit urinary bladder by stimulation of β -adrenoceptors as seen in human urinary bladder (20, 21), suggesting that β -adrenergic agonists promote the urine storage by increasing urinary bladder capacity.

Substantial pharmacological and physiological evidences indicate that urethral tone is mainly regulated by α -adrenoceptors (22). In the presence of timolol,

Urinary bladder

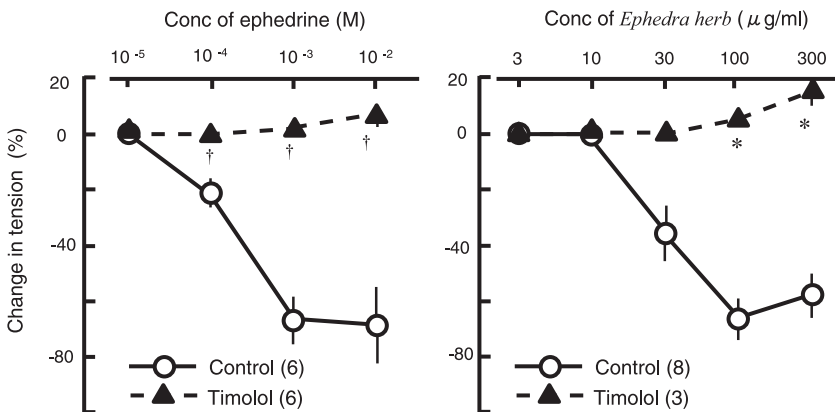


Fig. 1. Relaxant responses to ephedrine (left panel) and *Ephedra herb* (right) in female rabbit urinary bladder strips with (triangle) and without (circle) 10^{-6} M timolol partially contracted with prostaglandin (PG) $\text{F}_{2\alpha}$ ($0.2 - 1 \times 10^{-6}$ M). Relaxations induced by 10^{-4} M papaverine were taken as 100%. Numbers in parentheses indicate the number of experiments. Comparisons were made using the unpaired t -test. Bars = S.E.M. * $P < 0.05$, † $P < 0.005$, vs Control.

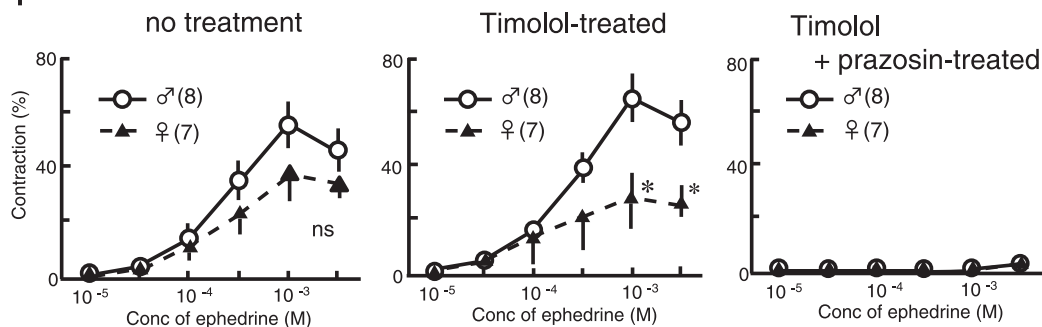
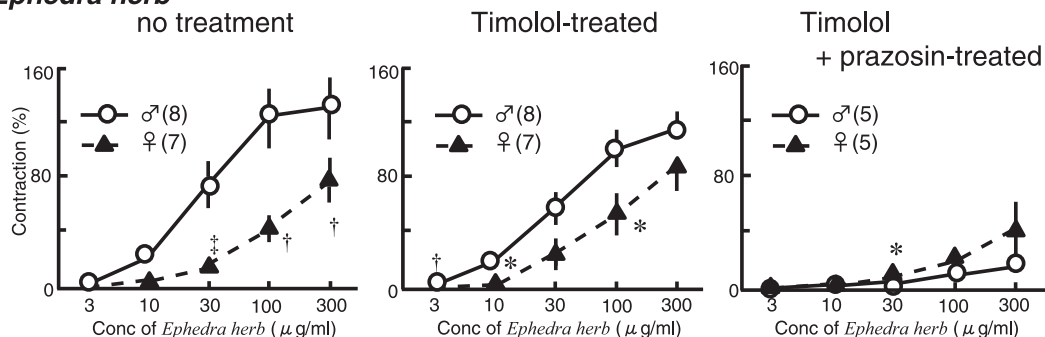
Ephedrine***Ephedra herb***

Fig. 2. Contractile responses to ephedrine (top panel) and *Ephedra herb* (bottom) in male (circle) and female (triangle) rabbit urethral strips without treatment (left), with 10^{-6} M timolol (center) and 10^{-6} M timolol plus 10^{-5} M prazosin (right). Contractions induced by 5 mM Ba^{2+} were taken as 100%. Numbers in parentheses indicate the number of experiments. Comparisons were made using the unpaired *t*-test. Bars = S.E.M. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.005$, vs male.

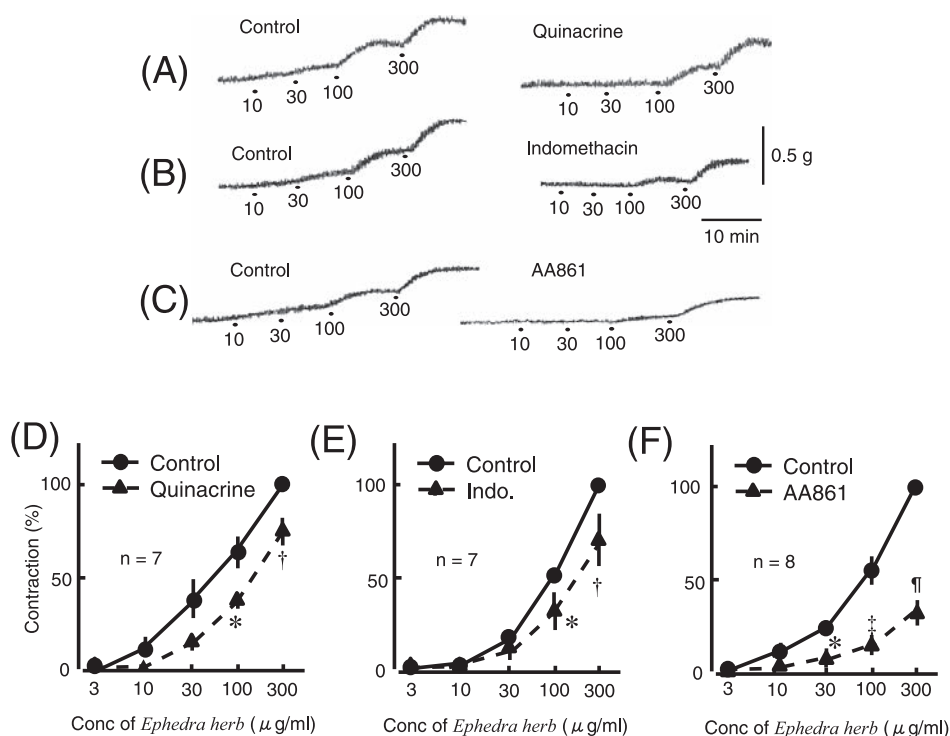


Fig. 3. Real tracings of the contractile responses to *Ephedra herb* in female rabbit urethral strips exposed to control media (Control, A, B, and C, left) and those containing 3×10^{-5} M quinacrine (A, right), 10^{-6} M indomethacin (B, right), or 10^{-5} M AA861 (C, right) in the presence of 10^{-6} M timolol plus 10^{-5} M prazosin. Contractile responses to *Ephedra herb* in female rabbit urethral strips without (circle) and with (triangle) 3×10^{-5} M quinacrine (D), 10^{-6} M indomethacin (E), or 10^{-5} M AA861 (F) in the presence of 10^{-6} M timolol plus 10^{-5} M prazosin. Contractions induced by 300 $\mu\text{g/ml}$ of *Ephedra herb* in the control media were taken as 100%. n, number of strips. Comparisons were made using the unpaired *t*-test. Bars = S.E.M. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.005$, †† $P < 0.0001$, vs Control.

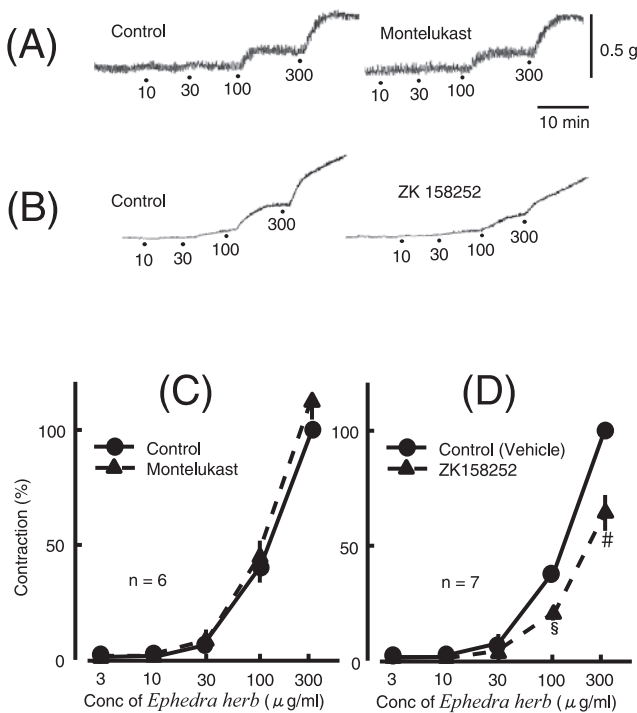


Fig. 4. Real tracings of the contractile response to *Ephedra herb* in female rabbit urethral strips exposed to control media (Control, A and B, left) and those containing 10^{-6} M montelukast (A, right) or 10^{-4} M ZK158252 (B, right) in the presence of 10^{-6} M timolol plus 10^{-5} M prazosin. Contractile responses to *Ephedra herb* in female rabbit urethral strips without (circle) and with (triangle) 10^{-6} M montelukast (C) or 10^{-4} M ZK158252 (D) in the presence of 10^{-6} M timolol plus 10^{-5} M prazosin. Contractions induced by 300μ g/ml of *Ephedra herb* in the control media were taken as 100%. n, number of strips. Comparisons were made using the unpaired *t* test. Bars = S.E.M. $^{\S}P < 0.001$, $^{\#}P < 0.0005$, vs Control.

ephedrine produced less contraction in the female than that in the male, and the contraction was abolished by treatment with prazosin, suggesting that functional α_1 -adrenoceptor populations in the female urethra may be less than those in the male. Thus, resistance to urine outflow may be weaker in the female than that in the male, when sympathetic nerves innervating the urethra are activated. In anesthetized animals, hypogastric nerve stimulation increased the intraurethral pressure, and the increase is blocked by α_1 -blockers (23). These findings provide a rationale for using α -adrenergic agonists to promote urine storage by increasing urethral resistance. Thus, ephedrine is considered to have a beneficial effect on SUI since it acts as both an α - and a β -adrenergic agonist. In addition, it enhances release of noradrenaline from sympathetic nerves. In fact, Diokno and Taub (10) reported that oral administration of ephedrine sulfate in doses ranging from 44–200 mg, daily, were effective for urinary incontinence in 27 out of 38 patients. However, some patients discontinued the drug after

only several days of treatment because of anxiety, hyperexcitability, and cardiac palpitation. Therefore, clinical use of ephedrine for treatment of SUI is not recommended because of the adverse effects.

Ephedra herb also contracted the urethral strips, and the contraction was more potent than that by ephedrine both in the male and female rabbits regardless of the β -adrenoceptor blockade (Fig. 2). Treatment with prazosin abolished the ephedrine-induced contraction, but not the *Ephedra herb*-induced contraction. Thus, *Ephedra herb* is found to produce contraction via a mechanism other than stimulation of α_1 -adrenoceptors. The contraction of the urethral strips was not affected by a sufficient concentration of atropine, yohimbine, methysergide, or chlorphenylamine under the blockade of α_1 - and β -adrenoceptors, suggesting that the contraction is not mediated via stimulation of muscarinic, α_2 , 5-HT₁ + 5-HT₂, or H₁ receptors. Quinacrine, indomethacin, and AA861 significantly inhibited the contraction, suggesting that cyclooxygenase metabolites and 5-lipoxygenase metabolites are involved in the response. In fact, prostaglandins have been reported to be a constricting agent in the rabbit urethra (24), but the involvement of 5-lipoxygenase metabolites have not been reported. In the present study, the contraction resistant to prazosin was inhibited by ZK 158252, a leukotriene (LT) B₄-receptor antagonist (18), but was not by montelukast, a CysLT₁-receptor antagonist, suggesting that LTB₄, but not LTC₄ or LTD₄, is involved in the response. Bouchelouche et al. (19) has reported that LTD₄ produced a contraction followed by an increase in $[Ca^{2+}]_i$, which was inhibited by montelukast, in human detrusor smooth muscles. However, receptor functions of leukotrienes in the urethra are not precisely known at this moment. It has been reported that LTB₄, known as a potent leukocyte chemoattractant, produced contractions in the guinea-pig trachea, bronchus, and pulmonary artery and in the human pulmonary artery (25). Taken together, *Ephedra herb* may contract the urethra by producing several metabolites of arachidonic acid besides by stimulating α_1 -adrenoceptors. The metabolites produced by 5-lipoxygenase probably stimulate receptors of LTB₄, but not of LTD₄.

As described earlier, Japanese gynecologists obtained a nice clinical result with little adverse effects when *Kakkon-to* was administered to the SUI female patients. The dosage of the herbal medicine used was 5.0–7.5 g daily. *Kakkon-to* contains 16.7% of *Ephedra herb*, and *Ephedra herb* contains less than 1.0% of ephedrine and some other α_1 -adrenoceptor stimulants such as phenylpropanolamine, pseudoephedrine, and *N*-methyl-ephedrine. If this is the case, the effective dose of *Kakkon-to* for treatment of SUI contains 13.0 mg

ephedrine at most, and the adverse effect of ephedrine, which was reported to be induced at doses higher than 40 mg daily (10), may not be induced. In the present study, *Ephedra herb* is found to be effective for SUI by mechanisms other than those that ephedrine and other α_1 -adrenoceptor stimulants elicit. In this context, *Ephedra herb* itself might be a possible therapeutic as long as proper doses are used.

Since *Kakkon-to* contains other substances than *Ephedra herb*, it is difficult to fully explain the clinical merit of *Kakkon-to* for urinary incontinence. However, *Ephedra herb* itself is difficult to use for the treatment because of the adverse reaction. Therefore, the mechanism associated with arachidonic acid metabolites may be important to create a new strategy for the treatment of SUI.

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References

- Thom D. Variation in estimates of urinary incontinence prevalence in the community: effects of differences in definition, population characteristics, and study type. *J Am Geriatr Soc*. 1998;46:473–480.
- Burgio KL, Locher JL, Goode PS. Combined behavioral and drug therapy for urge incontinence in older women. *J Am Geriatr Soc*. 2000;48:370–374.
- Weiss BD. Selecting medications for the treatment of urinary incontinence. *Am Fam Physician*. 2005;71:315–322.
- Salmon UJ, Walter RL, Geist S. The use of estrogen in the treatment of dysuria and incontinence in post-menopausal women. *Am J Obstet Gynecol*. 1941;42:845.
- Fantl JA, Bump RC, Robinson D, McClish DK, Wyman JF. Efficacy of estrogen supplementation in the treatment of urinary incontinence. The Continence Program for Women Research Group. *Obstet Gynecol*. 1996;88:745–749.
- Castro-Diaz D, Amoros MA. Pharmacotherapy for stress urinary incontinence. *Curr Opin Urol*. 2005;15:227–230.
- Ozaki Y. Studies on antiinflammatory effect of Japanese Oriental medicines (kampo medicines) used to treat inflammatory diseases. *Biol Pharm Bull*. 1995;18:559–562.
- Corcos J, Beaulieu S, Donovan J, Naughton M, Gotto M. Symptom Quality of Life Assessment Committee of the First International Consultation on Incontinence: Quality of life assessment in men and women with urinary incontinence. *J Urol*. 2002;168:896–905.
- Westfall TC, Westfall DP. Adrenergic agonists and antagonists. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's the pharmacological basis of therapeutics, 11th ed. New York: McGraw-Hill; 2006. p. 237–295.
- Diokno AC, Taub M. Ephedrine in treatment of urinary incontinence. *Urology*. 1975;5:624–625.
- Toda N, Hatano Y, Hayashi S. Modifications by stretches of the mechanical response of isolated cerebral and extracerebral arteries to vasoactive agents. *Pflugers Arch*. 1978;374:73–77.
- Kishimoto T, Aoki K, Okamiya Y, Takeshita T, Naruchi T. Effects of clenbuterol on resting tension and contractile response in vesicourethral smooth muscle of rabbits. *Jpn J Smooth Muscle Res*. 1989;25:13–25.
- Kimoto Y, Nozaki M, Itoh T. Actions of the alpha-1 adrenoceptor blocker bunazosin on the norepinephrine-induced contraction of smooth muscles in the rabbit proximal urethra. *J Pharmacol Exp Ther*. 1987;241:1017–1022.
- Hashimoto S, Kigoshi S, Muramatsu I. Neurogenic responses of urethra isolated from the dog. *Eur J Pharmacol*. 1992;213:117–123.
- Okamura T, Yamazaki M, Toda N. Responses to histamine and acetylcholine in isolated monkey mesenteric veins versus arteries. *Cardiovasc Res*. 1994;28:667–672.
- Juan H, Sametz W. Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed: mode of action. *Naunyn Schmiedeberg Arch Pharmacol*. 1980;314:183–190.
- Yoshimoto T, Yokoyama C, Ochi K, Yamamoto S, Maki Y, Ashida Y, et al. 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadienyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. *Biochim Biophys Acta*. 1982;713:470–473.
- Matousek M, Mitsube K, Mikuni M, Brännström M. Inhibition of ovulation in the rat by a leukotriene B₄ receptor antagonist. *Mol Hum Reprod*. 2001;7:35–42.
- Bouchelouche K, Andersen L, Nordling J, Horn T, Bouchelouche P. The cysteinyl-leukotriene D₄ induces cytosolic Ca²⁺ elevation and contraction of the human detrusor muscle. *J Urol*. 2003;170:638–644.
- Anderson KE. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. *Pharmacol Rev*. 1993;45:253–308.
- Nomiya M, Yamaguchi O. A quantitative analysis of mRNA expression of alpha 1 and beta-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. *J Urol*. 2003;170:649–653.
- Yamaguchi T, Kitada S, Osada Y. Role of adrenoceptors in the proximal urethral function of female and male rabbits using an in vivo model of isovolumetric pressure generation. *Neurourol Urodyn*. 1993;12:49–57.
- Willette RN, Sauermeier CF, Hieble JP. Role of alpha-1 and alpha-2 adrenoceptors in the sympathetic control of the proximal urethra. *J Pharmacol Exp Ther*. 1990;252:706–710.
- Sudoh K, Inagaki O, Honda K. Responsiveness of smooth muscle in the lower urinary tract of rabbits to various agonists. *Gen Pharmacol*. 1997;28:629–631.
- Sakata K, Dahlen SE, Back M. The contractile action of leukotriene B₄ in the guinea-pig lung involves a vascular component. *Br J Pharmacol*. 2004;141:449–456.