表3● 健康食品の安全性に影響する要因

要因	備考
製品の品質，偽装表示	重金属，細菌などの有害成分の混入。製品の表示と内容物が異なる場合，また偽装表示が行われている場合は，既知の科学的根拠が適用できなくなり，製品の安全・安心な利用の障害となる。
利用方法	長期間，大量に，また本来の目的とは異なった利用。食経験があったとしても，摂取量や摂取期間，調理方法が異なる場合は安全とはいえない。健康食品が病気の治療や治癒の目的で利用された条件では，健康被害が発生する確率は高くなる。
利用対象者	ハイリスクグループ（高齢者，幼児，妊婦，アレルギー体質，病者）の利用。安全性の検討が実施されていたとしても，その対象はほとんど健常人であり，ハイリスクグループでの影響は不明。
同時に摂取している医薬品や他の健康食品との相互作用	医薬品と併用することにより，医薬品の作用の増強あるいは減弱が起こる。複数の健康食品を摂取することにより，成分間の相互作用が起こり，有害な影響が発現する可能性がある。
不確かな情報の氾濫	科学的根拠のない情報により，製品の乱用が行われる。病者が，病気の治療・治癒目的に利用することにより，病気が進行したり悪化したりする可能性がある。

1）製品の品質や偽装表示

具体的には，違法な医薬品成分の添加，有害物質の混入，表示と実際の内容物の違いなどである。偽装表示の場合は，原材料が高価な時や需要が高くて入手が困難な条件で起きているようである[3]。もし健康食品に偽装表示や違法な医薬品成分の添加が行われていなかったならば，過去の重篤な被害はほとんど起こらなかったであろう。製品の品質が一定していないこと，表示と内容物が異なることは，健康食品の特徴ともいえる事項で，医薬品との決定的な違いでもある。

2）利用方法

科学的根拠がない状況で，病者が病気の治療・治癒の目的で健康食品を利用すると被害を受けてしまう。また，漫然と長期間利用することも問題である。近年，民間療法として利用されてきたハーブが話題になっているが，本来の民間療法におけるハーブの利用は，期待する症状の緩和を目的として，数日あるいは長くても数週間の利用である。しかし，最近は科学的根拠もなく，伝統的に利用されてきたという単なるイメージで，1年から数年といった長期間の利用が行われている。そのような利用法は健康被害につながると考えられる。

3）利用者側の体質

ハイリスクグループ（例えば，高齢者，妊婦，小児，病者）が利用すると，健康被害が起こる可能性が高い。アレルギーは体質による影響の受けやすさの典型的な事例である。健康食品の安全性がヒトにおいて検討されていたとしても，その対象者はほとんどが健常者であり，病者を対象とした検討ではない。つまり，病者が健康食品を

利用した時，どのような悪影響が発現するかは全くわからないのである。この点からも，健康食品を病気の治療・治癒の目的で利用することは，基本的には間違っているといえる。

4）医薬品と健康食品の相互作用

健康食品の利用者の中には医薬品を用いた治療を行っている人も少なくない。そのような条件では，医薬品と健康食品の相互作用が危惧される。健康食品と医薬品の併用により，医薬品の効果が減弱あるいは増強され，それが健康被害につながるのである。セントジョーンズワートについては，医薬品との相互作用が多くの研究で示されている[4]。しかし，他の健康食品素材と医薬品との相互作用は，ほとんどわかっていない。たとえ相互作用を実証した報告が多くあったとしても，それは原材料の情報である。また，健康食品には複数の素材が添加され，しかもその含有量も明確にはなっていない。そのため健康食品の最終製品として，医薬品との相互作用の有無を正確に判断することは現実的には極めて難しい状況になっている。

5）不確かな情報の氾濫

不確かな情報は，利用者側，利用方法，医薬品との相互作用など，他の要因に大きく影響する。にがり，雪茶，白インゲン豆などの事例のように，特にダイエット関連における情報では，何度も問題が起きている。現在のように健康食品が乱用されるようになったのも，テレビやインターネットを介して不確かな情報が氾濫しているからである。

最新の健康食品の安全性に関連した問題

健康食品が関連した安全性に関する最新の動向を知っておくことは，近い将来に起こりうる健康被害を予知し，回避する上で参考になる。「健康食品」の安全性・有効性情報（http://hfnet.nih.go.jp/）では，国内外から出された健康食品が関連した安全情報・被害関連情報を掲載している。そこで2006年6月から2007年7月までに掲載されている97件の情報を調べたところ，その内訳はシルデナフィルなどの強壮・強精に関連した医薬品成分の添加情報が51件，シブトラミンなどの肥満抑制に関連した医薬品成分の添加情報が17件，糖尿病治療薬のグリベンクラミド等の添加情報が6件，ステロイドの添加情報が6件，有害なハーブ類に関する情報が5件，その他として自然食品と標榜した製品に対する細菌や重金属の混入に関する情報などが数件であった。

このデータから，違法に医薬品成分が添加されている製品がかなり多く，しかも約50％は強壮・強精に関連した内容，20％はダイエット関連となっていることがわかる。強壮・強精やダイエット関連の製品は，インターネットを介して販売されているケースが多い。すなわちインターネットを介してそのような製品を購入することは，健康被害を受ける危険性が極めて高いことを示しているといえる。

Point

健康食品による被害を防ぐために利用者は，インターネット等を介した製品を安易に入手して利用しないこと，基本的に健康食品は不足している栄養成分等の補給・補完を目的に利用し，病気の治療・治癒目的には利用しないことである。最近の調査によると，健康食品等の利用目的として，健康の維持や栄養成分補給が多いが，病気の

治療目的に利用している人も少なくないようである[5]。健康食品はあくまで食品であり，有効性・安全性の科学的根拠，安全で効果的な利用ができる環境，製品の品質といった点で明らかに医薬品とは異なる。病気の人が医薬品と健康食品を併用することは，品質のしっかりした医薬品にわざわざ混ぜ物をして服用しているようにも思える。

一方，製造・販売業者は，明確な根拠もなく良いといわれている複数の成分を製品に添加したり，品質が保証されていないような製品を流通させたりするべきではない。そのような製品は，医薬品との相互作用の有無，ならびに健康被害が発生した時の正確な原因究明の障害になる。品質を確保するためにはGMP基準で製品を製造する取り組みが必要である。最近，国内外でそのような取り組みが行われ始めている。品質が信頼できる製品は，違法に医薬品成分が添加された製品と明確に区別できる。

健康食品が直面している課題に対応するためには，消費者に健康食品の有効性だけでなく，同時に利用における危険性について正しく情報提供することが求められる。その際，危険性情報は過剰な不安感を抱かせないようにするため，「誰が，何を，どれだけの量と期間で利用し，どのような有害な症状を呈したか」という，具体的情報として提供するべきである。情報が具体的であれば，消費者がその内容を正しく把握できるようになり，健康被害の発生はある程度防止できるであろう。上記の事項を実施する上で，医療ならびに製造・流通に従事する栄養学，薬学，医学等の専門職の益々の活躍が期待される。

プロフィール

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A Randomized Controlled Study on the Effects of Gargling with Tea Catechin Extracts on the Prevention of Influenza Infection in Healthy Adults

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Experimental studies have revealed that tea catechins prevent influenza virus infection ; however, the clinical effects have been inconclusive. At the onset of the influenza season, a randomized, double-blind, placebo-controlled study was conducted from December 2005 to March 2006 in Japan. A total of 404 healthy volunteers, 20-65 years of age, were enrolled and randomly assigned to two groups : the catechin group gargling with tea catechin extract solution (approximately 400 μg/mL catechins) or the placebo group gargling without tea catechin extracts. In both groups, gargling was performed three times daily for 90 days. All participants were inoculated with the influenza vaccine before participating in the study. The primary outcome measure was the incidence rate of influenza infection during the study identified by a rapid assay for influenza virus antigens. On an intention to treat basis, 195 participants in the catechin group and 200 in the placebo group who started the intervention were included in the analysis. Of the participants, 6 (1.5%) were infected with influenza. The incidence rate of influenza infection in the catechin group (1.0%, 2 participants) was half that in the control group (2.0%, 4 participants), but not significant between the two groups. We could not find significant effects of gargling with tea catechin on prevention of influenza in the healthy adults inoculated with the influenza vaccine of the 2005-2006 season. However, the effects in more susceptible groups, i. e., those not vaccinated against the influenza virus, children, elderly or immunosuppressed people remain inconclusive.

Trial registration : ClinicalTrials.gov ID, NCT00239213

Key words : catechin, gargling, influenza infection, prophylaxis, healthy adults

Introduction

Influenza virus infection occurs as both a pandemic and interpandemic, and in all age groups worldwide[1,2]. In order to reduce morbidity and mortality, several potential strategies such as vaccination, antiviral medication, and hygienic procedures are in place. Vaccines are the most widely used for the prophylaxis of influenza infection, but the uptake of immunization varies substantially and vaccine supply continues to be a problem[3]. Thus far, evidence for the effects of antivirals, such as amantadine or neuraminidase inhibitor on the prophylaxis of influenza infection has not been established[4]. Upper respiratory tract infections are also the most common source of morbidity worldwide, and they are reported to be expensive for the healthcare systems[5,6]. Therefore, it is important to find ways to reduce the frequency of both influenza infection and common colds.

Catechins are the major components of tea flavonoids. Experimental studies have demonstrated that catechins possess various physiological activities such as antioxidative, anticancer,

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hypolipidemic, hypoglycemic, hypotensive, antibacterial, and antiviral effects[7,8]. *In vitro* studies have revealed that tea catechin extracts prevent influenza virus infection and act against a few respiratory tract viruses, such as respiratory syncytial virus (RSV), parainfluenza virus, or adenovirus[9-16]. However, apart from those in the Japanese literature, few reports are available on the clinical effects of gargling with catechins on the prevention of influenza or upper respiratory tract infections[17-19]. Recently, in a small prospective cohort study, we reported that gargling with tea catechin extracts was effective in preventing influenza infection in elderly nursing home residents[19]. Based on this background, we designed a randomized, double-blind study to evaluate the effects of gargling with catechin extracts on the prevention of influenza infection.

Methods

1. Study participants

The study was conducted as a randomized, double-blind, placebo-controlled trial, for 90 days at the onset of the influenza season from October 2005 to May 2006. It was conducted in central Japan in three cities, namely, Shizuoka, Hamamatsu, and Higashi-Murayama city. From the three study areas, 458 healthy adult volunteers were recruited for the study that was to be conducted from October 2005 to November 2005. The participants were initially screened by a self-administered questionnaire for inclusion and exclusion criteria. The criteria for inclusion comprised the following : either gender ; age, 20 to 65 years ; subjectively healthy, and vaccinated against influenza prior to participation in the study. The criteria for exclusion criteria comprised the following : gargling with liquids other than water during the study ; allergy to tea ; and medical conditions such as a low immune state (e.g., collagen diseases, poorly controlled diabetes, tuberculosis, HIV/AIDS, or cancer), an allergic state (e.g., bronchial asthma, severe hay fever, a severe hypersensitivity to food ingestion), or severe dysfunctions (cardiac, respiratory, renal, or hepatic). Further, participants were excluded by the study physician responsible for each area if they were on medication that would interfere with the evaluation, such as immunosuppressive drugs, corticosteroids, antibiotics, or other herbal products (e.g., echinacea, American ginseng) that may interfere with the study.

The self-administered questionnaire used to collect the participants' baseline characteristics including year of birth, gender, body mass index, smoking and alcohol consumption, and tea or health food consumption. Between late October and early December 2005, prior to participating in the study, all of the volunteers were vaccinated with an influenza vaccine from the same batch. In accordance with the recommendations of the World Health Organization (WHO) for the 2005-2006 northern hemisphere influenza season, the vaccine used in the study contained the following viral strains : A/New Caledonia/20/99(H1N1)-like virus, A/New York/55/2004 (H3N2)-like virus, and B/Shanghai/361/2002-like virus. The study was approved by the Ethics Committee of the University of Shizuoka and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants prior to conduction of the study.

2. Intervention

Participants were randomly selected to receive either the catechin extract or placebo. Randomization was performed using a scheme that was generated using the Statistical Analysis System (SAS) for Windows, version 9.1.3 (SAS institute, Inc., Cary, NC, USA). The randomization codes were not broken until all the data were analyzed.

Participants in both groups were instructed to gargle three times daily for 90 days from December 2005 to March 2006. The participants were instructed to dissolve 1.0 g of the tea catechin extract or placebo in half a cup (approximately 100 mL) of sterilized tap water and gargle for approximately 15 seconds, three times consecutively, thrice a day. The final concentration received by the catechin group was approximately 400 μg/mL catechins, which is the same as that of commercially sold common green tea beverages in Japan. The concentration of total catechins was determined by the *in vitro* study, to be a sufficient amount for the inhibition of the infectivity of influenza virus[9]. The catechins were formulated as polyphenon 70A (Mitsui Norin Co., Ltd., Tokyo), and the total catechin content was 82.8%, including 59.3% (−)-epigallocatechin gallate, 15.1% (−)-epicate-

chin gallate, 3.8% (−)-gallocatechin gallate, 1.8% (−)-epicatechin, 1.7% (−)-epigallocatechin, 0.5% (−)-catechin gallate, 0.3% (−)-gallocatechin, and 0.3% (−)-catechin. The placebo was formulated to be almost the same color and taste of catechins, and the quality of double-blinding was ascertained by the clinical research coordinators before the start of the study.

During the follow-up period, all of the participants received a 30-day supply of either the catechin extract or placebo, and they were requested to fill in the prescribed form (gargling diary) every day. The participants had to report the frequency of gargling and the severity of their cold-related symptoms including nasal (rhinorrhea and sneezing), pharyngeal (soreness and itching), bronchial (cough and phlegm), and general symptoms (feverishness, arthralgia, and malaise) scored on a 4-point scale according to the Jackson method (0=no symptoms, 1=mild symptoms, 2=moderate symptoms, and 3=severe symptoms)[20]. This assessment was further verified by the study physician of each area or clinical research coordinators via telephone or e-mail. Each symptom score was reported at the maximum severity of a cold event, and the total symptom score was calculated by adding the symptom scores. Participants were also asked to report any secondary complications, hospital admission, job absence, or adverse events. The study physician of each area attended to the adverse events and identified the causal relationship for each.

During the study of prophylaxis, all participants were asked not to take any other cold medication, gargle with povidone iodide or tea, or change their hygiene related habits such as hand-washing except for the necessity of use at the treatments. Every month, during the follow-up period, the study physician and clinical research coordinator from each area monitored the participants' health condition and compliance with gargling instructions and encouraged them to maintain the prescribed intervention.

Influenza infection was defined as certified by a commercially available rapid assay for influenza virus antigens. The assay could not distinguish between mere carriage of the virus and the presence of actual infection. Therefore, the assay was performed only if a participant had an influenza-like illness. The symptoms of an influenza-like illness were a minimum temperature of 37.8°C accompanied by a recent or aggravated cough and one or more of the following signs or symptoms : chills, myalgia, malaise, sore throat, new or increased rhinorrhea or headache, and loss of appetite or diarrhea. If an influenza infection or influenza-like illness was detected, antiviral therapy was administered based on the physician's decision. Upper respiratory tract infection was defined by the presence of cold-related symptoms but not influenza-like illness.

3. Outcome measures

With regard to the primary outcome, the incidence rates of influenza infection, detected by a rapid assay for influenza virus antigens, were measured, and the incidence of infection during the study was compared between the two groups. Secondary outcome measures comprised the incidence rates of upper respiratory tract infections, the severity of the symptoms and the duration of the cold among incident cases, and the incidence-free time for influenza or upper respiratory tract infections after the intervention. The outcomes were assessed with a self-administered questionnaire, and the participants who suffered from influenza or upper respiratory infections on the first day of intervention were excluded from the analysis. For the safety evaluation, adverse events such as throat and respiratory tract irritation and obstruction or allergic bronchial spasm were examined at each gargling session during the study.

All analyses were performed by a statistician under blinded conditions. The data for the participants who had been randomly assigned to a group were analyzed, and those who provided only baseline information on an intention-to-treat basis were excluded. Participants who withdrew or emigrated were censored (i. e., data were included in analysis up to the point of withdrawal, even though they did not stay for the complete observational period).

4. Statistical analysis

Our previous report demonstrated that the incidence of influenza in elderly nursing home residents was 2% when they gargled with catechin extract solution and 10% when gargling was not perfor-

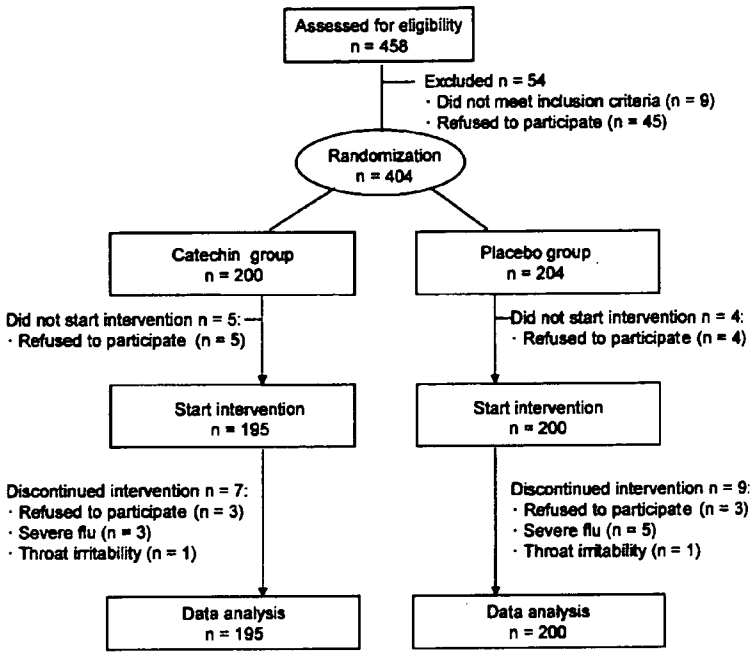


Fig. 1 Flowchart of the study

med. Consequently, the sample size was calculated as 190 for each group at a power level of 0.90, and a significance level of 0.05. Expecting 5% dropouts, we set the total sample size at 400.

All the statistical analyses were performed using SAS for Windows, version 9.1.3. The data for continuous variables was expressed as the mean± standard deviation (SD). Differences in quantitative data between the groups were assessed using the Student's *t*-test. The Fisher's exact test was used to compare the differences in the incidence rates of influenza or upper respiratory infections, and other qualitative data. The generalized Wilcoxon test was used to compare the differences in the incidence-free time of influenza or upper respiratory tract infections, and cumulative incidence rates at 30, 60, 90 days were determined by the Kaplan-Meier method. The Wilcoxon test was used to compare the differences in the ordinal data, such as the severity of symptoms and duration of the cold among the incident cases. A P value of less than 0.05 was used to indicate statistical significance.

Results

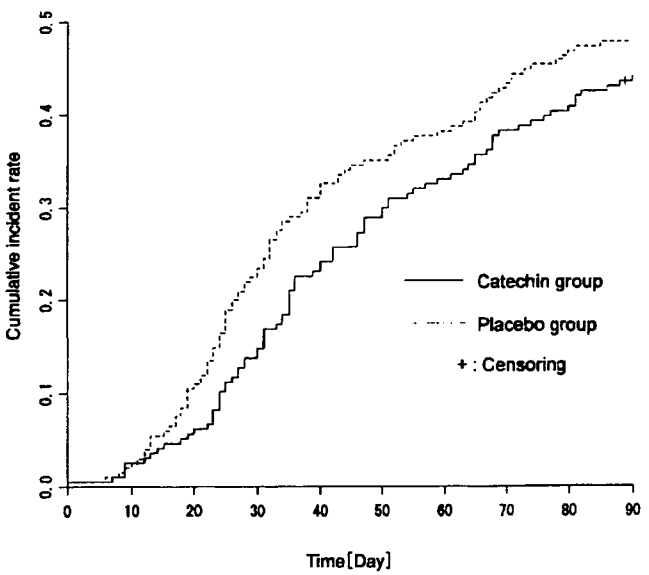
Figure 1 illustrates the flowchart of the study. After the screening process, a total of 404 volunteers (39.9±11.4 years, mean±standard deviation ; 88 men, 316 women) were enrolled and randomly assigned to the catechin or placebo group. Of these, 200 were assigned to receive the catechin extract and 204, to receive placebo. Of the participants in the catechin group, 5 did not start treatment, and in the placebo group, 4 did not start their treatment because of refusal to consent. The 195 participants in the catechin group and 200 in the placebo group who started intervention were included in the analysis on an intention-to-treat basis. Treatment was discontinued for 7 participants in the catechin group and 9 in the placebo group for various reasons such as refusal to participate (6 participants), severe flu (8 participants), or throat irritation (2 participants).

The baseline characteristics of the participants are shown in Table 1. Between the two groups, there were no significant differences in age, gender, body mass index, smoking and alcohol consumption, and tea and health food consumption. Although all the participants received an influenza vaccine, 1.5% of the participants (6 participants) were infected with influenza that was identified as type A using the antigen assay. No influenza pandemic or related use of antiviral prophylaxis occurred during the study.

Table 1 Baseline characteristics of the participants in the group gargling with tea catechin extracts solution (catechin group) and the placebo (control) group*

	Catechin group (N=200)	Control group (N=204)	*p* value
Age (years)	39.6±11.4	40.2±11.5	0.5849
Men/Women	36/164	52/152	0.0718
Body mass index†	21.1±2.77	21.5±2.77	0.1476
Smoking (+/past/−)	39/12/149	47/14/143	0.6147
Alcohol (+/past/−)	117/4/79	114/1/89	0.3080
Tea drinking (mL/day)	607±333	591±341	0.6471
Taking health foods (+/−)	33/167	41/159	0.5820

*Plus-minus values are means±SD. There were no significant differences between the groups.
†The body mass index is the weight in kilograms divided by the square of the height in meters.



Kaplan-Meier estimate [95% confidence interval] of cumulative incident rate

	Catechin group	Placebo group
Cumulative incident rate at 30 day	0.1341[0.0861-0.1820]	0.2350[0.1763-0.2938]
Cumulative incident rate at 60 day	0.3270[0.2607-0.3933]	0.3820[0.3144-0.4495]
Cumulative incident rate at 90 day	0.4374[0.3662-0.4923]	0.4790[0.4094-0.5486]

p-value: 0.1720[Generalized Wilcoxon test]

Fig. 2 Kaplan-Meier incidence curves of influenza or upper respiratory tract infections according to intervention

The incidence rate of influenza infection in the catechin group (1.0%, 2 participants) was half that in the placebo group (2.0%, 4 participants), but no significant difference was observed between the two groups. The incidence rate of upper respiratory infections was also not significantly different between the two groups (48.2%, 94 participants in the catechin group vs. 51.5%, 103 participants in the placebo group). Figure 2 shows the cumulative incidence-free time curves of influenza or upper respiratory infections in each group. The incidence-free time was not significantly different (p value, 0.1720). As determined by the Kaplan-Meier method, the cumulative incidence rate at 30, 60, and

Table 2 The comparison of the incidence rates of influenza and respiratory tract infection, severity and duration of the respiratory tract infection between the group gargling with tea catechin extract solution (catechin group) and the placebo (control) group

	Catechin group (N=195)	Control group (N=200)	*p* value
Influenza illness	2 (1.0%)	4 (2.0%)	0.8423
Influenza-like illness	1	1	
Upper respiratory infections	94 (48.2%)	103 (51.5%)	0.4515
(1 time)	64	76	
(2 times)	26	20	
(3 times)	4	7	
Severity (total Jackson scores)			0.8934
(1-5)	31	37	
(6-10)	44	46	
(10<)	19	20	
Duration			
Median (Quartile)	7 (11)	9 (10)	0.1886
(Min.- Max.)	(2-55)	(2-41)	

90 days was 23.5%, 38.2%, and 47.9%, respectively, in the placebo group. On the other hand, this value was 13.4%, 32.7%, and 43.7% respectively, in the catechin group. The severity of the symptoms and duration of the cold among incident cases did not differ significantly between the two groups (Table 2).

In each group, there were no severe complications such as bronchopneumonia or encephalitis and no cases that required hospital admission. No significant differences in job absence were observed between the groups. The interventions were well tolerated by the participants. Only 2 participants experienced an adverse event, i.e., mild throat irritation. The frequency of throat irritation was similar in the 2 groups (0.5% in each group), and the symptoms disappeared after discontinuation of the gargling despite the continued consumption of tea. No serious adverse events such as respiratory tract irritation and obstruction or allergic bronchial spasm were observed during the study.

Discussion

The present study is the first randomized, double-blind, placebo-controlled study to investigate the effects of gargling with tea catechin extracts on the prevention of influenza infection in healthy adults. Contrary to a previous report on elderly nursing home residents[19], we could not confirm that the positive effects of catechin extracts on the prevention of influenza infection in healthy adults inoculated with the influenza vaccine. However, the discrepancy in the results of the two studies might be due to the low incidence rate of influenza infection in our study (1.5%) that was insufficient to obtain the required statistical power. In a typical epidemic season, approximately 5%-15% of adults and children develop symptomatic influenza[21]. The 2005-2006 season that we studied was interpandemic in the United States as well as in Japan, and no remarkable pandemic outbreak occurred also in the study areas[22,23]. Further, the selection of healthy adults inoculated with influenza vaccine lowered the frequency of influenza infection. The rate of influenza infection is higher in people not vaccinated against the influenza virus and in children, elderly, or immunosuppressed people[24,25]. Therefore, it can be speculated that the incidence of influenza infection is higher if the study population comprised people who are highly vulnerable to influenza, and hence, the results would vary.

In experimental studies, catechins bind to the hemagglutinin of the influenza virus, and they inhibit viral adsorption to Madin-Darby canine kidney (MDCK) cells ; these results provide an insight into the mechanisms by which tea catechin extracts inhibit the influenza virus[9-14]. Although evidence from basic experiments is accumulating, data from randomized, controlled clinical trials

that are linked to the basic experimental results are not yet established. Further studies are required to clarify the effects of tea catechins in humans in order to recommend their use against influenza infection.

As shown in the results, we did not observe any significant effects of catechin extracts on the prevention of upper respiratory tract infections, although the cumulative incidence rate in the catechin group was slightly lower than that in the control group. In Japan, gargling is generally recommended as a preventive modality for upper respiratory tract infections[26]. Recently, Satomura et al reported that mere gargling with water was effective in preventing upper respiratory tract infections compared to the usual hygienic care[27]. Therefore, it should be considered that gargling itself has a placebo effect which is similar to that of gargling with tea catechin extracts.

Tea catechins are reported to be well tolerated, except in tea factory workers with occupational asthma induced by the inhalation of green tea dust[28-30]. During the three months of gargling, no serious side effects were observed in the participants, except for throat irritation in 0.5% of the participants in each group. The symptoms disappeared after discontinuation of gargling despite the continued consumption of tea. Therefore, the adverse effects were believed to be related to the gargling itself, not to catechins.

In summary, we could not find significant effects of gargling with tea catechin on the prevention of influenza or upper respiratory tract infections in the healthy adults who had been inoculated with the influenza vaccine. However, the effect on more susceptible groups, i.e., those not vaccinated against influenza, children, elderly, or immunosuppressed people remain inconclusive.

Acknowledgments

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Muscarinic and Alpha 1-Adrenergic Receptor Binding Characteristics of Saw Palmetto Extract in Rat Lower Urinary Tract

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OBJECTIVES To elucidate the in vitro and ex vivo effects of saw palmetto extract (SPE) on autonomic receptors in the rat lower urinary tract.

METHODS The in vitro binding affinities for alpha 1-adrenergic, muscarinic, and purinergic receptors in the rat prostate and bladder were measured by radioligand binding assays. Rats received vehicle or SPE (0.6 to 60 mg/kg/day) orally for 4 weeks, and alpha 1-adrenergic and muscarinic receptor binding in tissues of these rats were measured.

RESULTS Saw palmetto extract inhibited specific binding of [^{3}H]prazosin and [N-methyl-^{3}H]scopolamine methyl chloride (NMS) but not alpha, beta-methylene adenosine triphosphate [2,8-^{3}H]tetrasodium salt in the rat prostate and bladder. The binding activity of SPE for muscarinic receptors was four times greater than that for alpha 1-adrenergic receptors. Scatchard analysis revealed that SPE significantly reduced the maximal number of binding sites (B_{max}) for each radioligand in the prostate and bladder under in vitro condition. Repeated oral administration of SPE to rats brought about significant alteration in B_{max} for prostatic [^{3}H]prazosin binding and for bladder [^{3}H]NMS binding. Such alteration by SPE was selective to the receptors in the lower urinary tract.

CONCLUSIONS Saw palmetto extract exerts significant binding activity on autonomic receptors in the lower urinary tract under in vitro and in vivo conditions. UROLOGY 69: 1216–1220, 2007. © 2007 Elsevier Inc.

In many European countries phytotherapeutic agents, including saw palmetto extract (SPE), are widely used for the treatment of benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS), and these herbal remedies represent up to 80% of all drugs prescribed for these disorders.[1,2] Some clinical trials support the efficacy of SPE in improving symptoms associated with BPH and LUTS.[3–5]

Numerous mechanisms of SPE have been proposed, including inhibition of 5 alpha-reductase, antiandrogenic effects, antiproliferative effects, anti-inflammatory effects (inhibition of cyclo-oxygenase and lipoxygenase), and anti-edema effects.[6] However, these pharmacologic actions of SPE were observed by using relatively high concentrations or large dosages.[7,8] Therefore, it is uncertain whether the reported modes of action of SPE are therapeutically relevant.[3,4] Gutierrez *et al.*[9] showed relatively potent smooth muscle relaxant activity by SPE in isolated tissues, such as the urinary bladder of guinea pigs and rats. Furthermore, our recent study revealed that intraduodenally administered SPE significantly improves urodynamic symptoms in hyperactive bladder of rats by increasing bladder capacity and prolonging micturition interval.[10] Such improvement of urodynamic symptoms by SPE has been suggested to arise partly from its significant antagonism of autonomic receptors in the lower urinary tract.[10,11] The aim of this study was to clarify the in vitro and ex vivo effects of SPE on autonomic (alpha 1-adrenergic, muscarinic, and purinergic) receptors involved in lower urinary function.

MATERIAL AND METHODS

Materials

Saw palmetto extract (*Serenoa repens* purified extract) was provided by Indena Japan (Tokyo, Japan). Saw palmetto extract was obtained with hypercritical CO_2 (SABAL SELECT, Indena S.p.A., Milan, Italy). This extract contained high-molecular-weight compounds, which are esters of long chain alcohol with fatty acid. Saw palmetto extract was suspended in 0.5% methylcellulose. [N-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS, 2.997 TBq/mmol), [7-methoxy-^{3}H]prazosin (2.979

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TBq/mmol), (+)-[^{3}H]PN 200-110 (3.180 TBq/mmol), and 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, Mass). All other chemicals were purchased from commercial sources.

Animals and Administration of SPE

Male Sprague-Dawley rats weighing 250 to 350 g were purchased from SLC (Hamamatsu, Japan). Rats were housed with a 12-hour light–dark cycle and fed laboratory food and water ad libitum. Rats received SPE (0.6, 6, and 60 mg/kg/day) by gavage once per day for 4 weeks. The control animals received vehicle alone. The repeated SPE administration had little effect on the eating and drinking behaviors and on the body weight of rats. This study was done according to guidelines approved by the experimental animal ethics committee of the University of Shizuoka.

Binding Assay of [^{3}H]Prazosin, [^{3}H]NMS, and [^{3}H]Alpha, Beta-MeATP

The radioligand binding assays for alpha 1-adrenergic, muscarinic, and purinergic receptors were performed using [^{3}H]prazosin,[12] [^{3}H]NMS,[13] and [^{3}H]alpha, beta-MeATP,[14] respectively. The bladder, prostate, submaxillary gland, heart, and spleen were dissected from rats under temporary anesthesia, and the tissues were minced with scissors. In the case of binding assays of [^{3}H]prazosin, each tissue was homogenized by a Kinematica Polytron homogenizer (Kinematica, PT 10-35) in ice-cold 50 mmol/L Tris-HCl buffer (pH 7.5). The homogenates were then centrifuged at 40,000*g* for 20 minutes at 4°C. The pellet, after suspension in the cold buffer, was centrifuged further at 40,000*g* for 20 minutes at 4°C, and the resulting pellet was finally resuspended in the cold buffer to use in the radioligand binding assay. In the case of [^{3}H]NMS binding assay, the bladder and submaxillary gland were similarly homogenized in 30 mmol/L Na^+/N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (pH 7.5). The homogenate was then centrifuged at 40,000*g* for 20 minutes at 4°C. The suspension of the resulting pellet was used. In the case of [^{3}H]alpha, beta-MeATP binding assay, the bladder homogenate in 50 mmol/L Tris/HCl buffer was centrifuged at 2000*g* for 10 minutes at 4°C. The pellet suspension was further centrifuged at 2000*g* for 10 minutes at 4°C, and the supernatant was combined with the original supernatant. The supernatant was centrifuged at 48,000*g* for 20 minutes at 4°C, and the suspension of the resulting pellet was used.

In in vitro displacement experiments, the rat tissue homogenates were incubated with [^{3}H]prazosin (500 pmol/L, prostate and spleen), [^{3}H]NMS (460 pmol/L, bladder and submaxillary gland), and [^{3}H]alpha, beta-MeATP (4.5 nmol/L, bladder) in the presence of various concentrations (10 to 1000 μg/mL) of SPE. In in vitro and ex vivo saturation experiments, the rat tissue homogenates were incubated with [^{3}H]prazosin (0.03 to 0.5 nmol/L, prostate, submaxillary gland, heart, and spleen) and [^{3}H]NMS (0.06 to 1.0 nmol/L, bladder and submaxillary gland). Thus, Scatchard analysis with a full range of radioligands was conducted to estimate apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) values.

Incubation was carried out for 60 minutes at 25°C (4°C in [^{3}H]alpha, beta-MeATP). 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]prazosin, [^{3}H]NMS, and [^{3}H]alpha, beta-MeATP was determined experimentally from the difference between counts in the absence and presence of 10 μmol/L phentolamine, 1 μmol/L atropine, and 3 μmol/L alpha, beta-MeATP, respectively.

Data Analysis

The K_d and B_{max} for radioligands were estimated by Scatchard analysis of the saturation data.[15] The binding activities of SPE to each receptor were estimated by IC_{50} values, which are the concentrations of SPE necessary to displace 50% of specific binding of each radioligand. Statistical analysis of the data was performed by a one-way analysis of variance followed by Dunnett's test for multiple comparisons.

RESULTS

In Vitro Effects on Alpha 1-Adrenergic, Muscarinic, and Purinergic Receptors

Saw palmetto extract (50 to 200 μg/mL) inhibited specific [^{3}H]prazosin binding in the prostate and spleen (Fig. 1A), and their IC_{50} values were 169 ± 24 and 188 ± 47 μg/mL, respectively. Similarly, SPE (10 to 1000 μg/mL) inhibited specific [^{3}H]NMS binding in the bladder and submaxillary gland of rats in a concentration-dependent manner (Fig. 1B), and the IC_{50} values were 40.0 ± 4.1 and 52.3 ± 4.4 μg/mL, respectively. Thus, the inhibitory effect by SPE of bladder [^{3}H]NMS binding was significantly (4.2 times, $P < 0.001$) more potent than that of prostatic [^{3}H]prazosin binding. It had little effect on bladder [^{3}H]alpha, beta-MeATP binding (Fig. 1C).

Scatchard analysis revealed that SPE (150 μg/mL) significantly reduced B_{max} values for specific [^{3}H]prazosin binding in the rat prostate (55.3%), compared with the corresponding control value (Table 1). Similarly, in the presence of SPE (50 μg/mL), there was a significant decrease of B_{max} values for specific [^{3}H]NMS binding in the bladder (55.2%). There was little change in K_d values for specific binding of each radioligand in rat tissues, except for a significant (32.0%) decrease of the K_d value for prostatic [^{3}H]prazosin binding.

Effects of Repeated Oral Administration of SPE on Autonomic Receptors

Repeated oral administration of SPE brought about a significant increase in B_{max} values for specific [^{3}H]prazosin binding in the prostate and spleen of rats, compared with the corresponding control values (Table 2). The enhancement in B_{max} values was significant in the prostate (23.6% and 36.7%, respectively) at the dose of 6 and 60 mg/kg/day and in the spleen (26.1%) at the dose of 60 mg/kg/day. Thus, the enhancement in B_{max} values in the prostate was exerted by a relatively low dose (6 mg/kg/day) of SPE. In the submaxillary gland and heart, there was little change in [^{3}H]prazosin binding parameters, except a significant (30.5%) increase in the heart at the dose of 60 mg/kg.

After repeated oral administration of SPE (0.6, 6, and 60 mg/kg/day), there was a significant (31.8% to 41.2%)

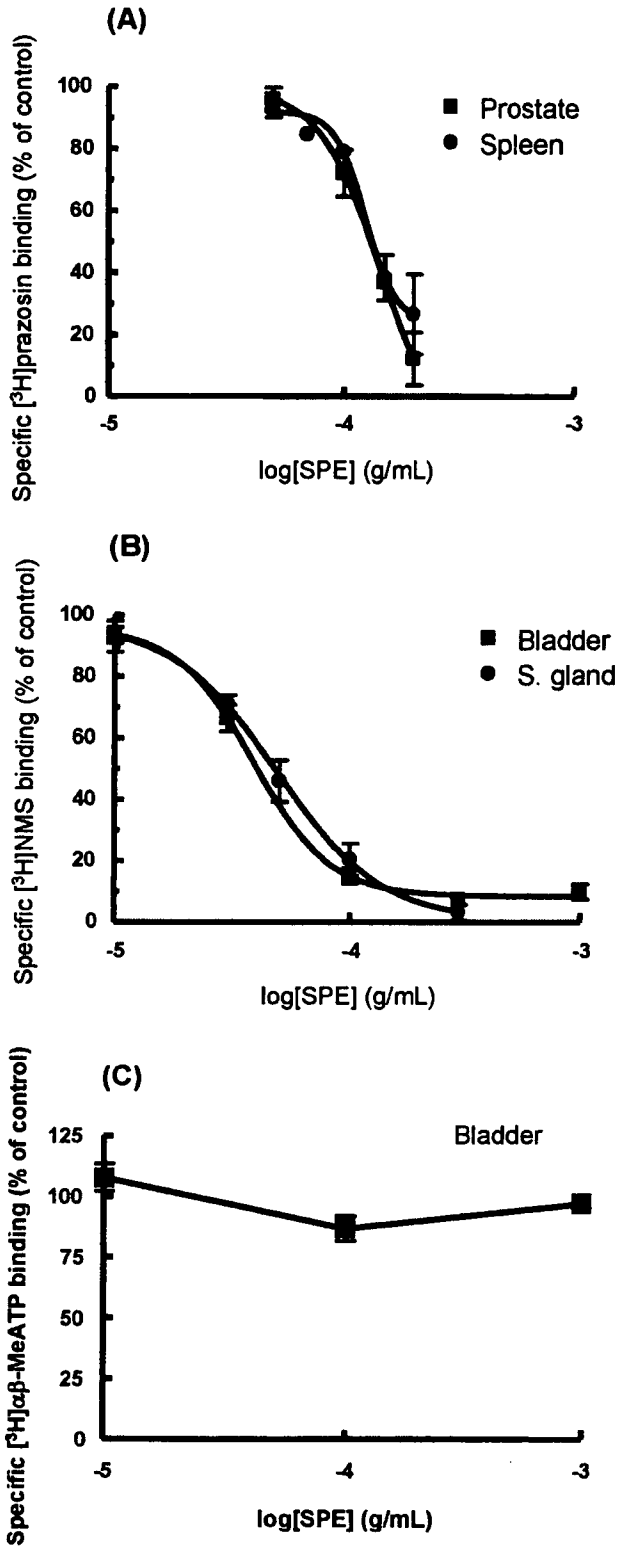

Figure 1. Inhibition by SPE of specific binding of **(A)** [^{3}H]prazosin, **(B)** [^{3}H]NMS, and **(C)** [^{3}H]alpha, beta-MeATP in rat tissues. Specific binding of [^{3}H]prazosin (500 pmol/L), [^{3}H]NMS (460 pmol/L), and [^{3}H]alpha, beta-MeATP (4.5 nmol/L) in rat tissues was measured in the absence and presence of four or five different concentrations (10 to 1000 μg/mL) of SPE. Each point represents the mean ± standard error of 5 to 8 ([^{3}H]prazosin), 8 to 9 ([^{3}H]NMS), and 3 ([^{3}H]alpha, beta-MeATP) determinations.

decrease of B_{max} values for specific [^{3}H]NMS binding in the rat bladder compared with the corresponding control value (Table 2). Similarly, the B_{max} value was significantly (17.9%) reduced in the submaxillary gland by SPE only at the high dose (60 mg/kg/day). Thus, the decrease of B_{max} values in the bladder compared with the submaxillary gland was seen by relatively low doses (0.6 and 6 mg/kg/day) of SPE. There was little change in K_d values for [^{3}H]NMS, except a slight (13.8%) increase in the submaxillary gland at the dose of 0.6 mg/kg.

COMMENT

In the majority of men with BPH and LUTS, the major goal of pharmacologic treatment is the relief of irritative and obstructive symptoms. These symptoms are effectively alleviated by alpha 1-adrenergic and muscarinic receptor antagonists. The in vitro and in vivo binding activities of SPE on autonomic receptors expressing in the rat lower urinary tract were examined. The administered oral doses of SPE were determined on the basis of in vivo pharmacologic doses in reducing detrusor contractility and number of micturition in rats.[10] In fact, it may be of pharmacologic significance that oral doses (0.6 to 60 mg/kg/day) of SPE were considerably lower than those previously reported for pharmacologic effects such as inhibition (100 to 640 mg/kg/day) of rat prostatic hyperplasia induced by hyperprolactinemia[16] and reduction (50, 100 mg/kg/day) of mast cell accumulation in the rat prostate.[17]

The in vitro experiment has shown that SPE inhibited specific binding of [^{3}H]prasozin and [^{3}H]NMS but not [^{3}H]alpha, beta-MeATP in the prostate, bladder, and other tissues of rats, in a concentration-dependent manner. According to IC_{50} values, the binding activity of SPE for muscarinic receptors was shown to be four times greater than that for alpha 1-adrenergic receptors. These receptor-binding affinities of SPE for autonomic receptors were nearly comparable to or greater than in vitro pharmacologic potencies of this extract (eg, inhibition of 5 alpha-reductase [IC_{50}: 71 μg/mL], anti-inflammatory effect [inhibition of cyclo-oxygenase and 5-lipoxygenase, IC_{50}: 28.1 and 18.0 μg/mL, respectively], and antiandrogenic effect [IC_{50}: 1004 μg/mL]) previously reported.[18,19] Furthermore, Scatchard analysis revealed that SPE caused a significant decrease of B_{max} values for specific binding of [^{3}H]prazosin and [^{3}H]NMS in the prostate and bladder of rats. Therefore, it could be presumed that SPE binds noncompetitively to alpha 1-adrenergic and muscarinic receptors in rat tissues.

To clarify whether SPE has some effects on neurotransmitter receptors under in vivo conditions, we investigated the effect of oral administration of SPE on autonomic receptors in the lower urinary tract of rats. Repeated oral administration of SPE produced a significant decrease of B_{max} values for specific [^{3}H]NMS binding in the rat bladder and submaxillary gland. Notably, such reduction in the number of [^{3}H]NMS binding sites was observed with relatively lower doses (0.6 and 6

Table 1. Effects of SPE on K_d and B_{max} for specific binding of [^{3}H]prazosin and [^{3}H]NMS in the rat prostate and bladder

Radioligands	Tissues	K_d (pmol/L)	B_{max} (fmol/mg protein)
[^{3}H]prazosin	Prostate		
	Control	59.2 ± 7.1	22.6 ± 1.8
	SPE (150 μg/mL)	40.3 ± 1.6*	10.1 ± 0.6†
[^{3}H]NMS	Bladder		
	Control	256 ± 77	192 ± 27
	SPE (50 μg/mL)	190 ± 14	86.1 ± 11.7*

SPE = saw palmetto extract; K_d = dissociation constant; B_{max} = maximal number of binding sites; [^{3}H]NMS = [N-methyl-^{3}H]scopolamine methyl chloride.
Values are mean ± standard error of 3 rats.
* $P < 0.05$ vs. control.
† $P < 0.01$ vs. control.

Table 2. K_d and B_{max} for specific binding of [^{3}H]prazosin and [^{3}H]NMS in rat tissues after repeated oral administration of SPE at doses of 0.6, 6, and 60 mg/kg

Organs	Dose (mg/kg)	K_d (pM)	B_{max} (fmol/mg protein)
Specific [^{3}H]prazosin binding			
Prostate	Control	49.7 ± 2.4	26.7 ± 0.5
	0.6	43.1 ± 2.0	30.1 ± 1.6
	6	51.9 ± 4.8	33.0 ± 1.3*
	60	54.6 ± 2.8	36.5 ± 1.2†
Submaxillary gland	Control	53.7 ± 3.0	165 ± 8
	0.6	50.7 ± 2.5	160 ± 5
	6	54.8 ± 2.2	162 ± 7
	60	60.6 ± 2.1	164 ± 8
Spleen	Control	26.4 ± 2.8	68.3 ± 4.0
	0.6	28.8 ± 2.2	75.1 ± 3.8
	6	27.5 ± 1.5	65.7 ± 5.1
	60	30.1 ± 1.2	86.1 ± 4.3†
Heart	Control	26.6 ± 1.6	68.7 ± 4.4
	0.6	27.1 ± 1.4	72.4 ± 6.9
	6	26.0 ± 1.5	77.2 ± 4.8
	60	34.7 ± 1.4*	77.3 ± 1.9
Specific [^{3}H]NMS binding			
Bladder	Control	157 ± 10	144 ± 12
	0.6	127 ± 13	84.7 ± 8.1†
	6	135 ± 11	98.2 ± 3.8†
	60	137 ± 5	97.5 ± 2.7†
Submaxillary gland	Control	98.4 ± 3.8	145 ± 3
	0.6	112 ± 3†	149 ± 10
	6	101 ± 2	149 ± 8
	60	105 ± 2	119 ± 2*

K_d = dissociation constant; B_{max} = maximal number of binding sites; [^{3}H]NMS = [N-methyl-^{3}H]scopolamine methyl chloride; SPE = saw palmetto extract.
Values are mean ± standard error of 3 to 7 rats.
* $P < 0.01$ vs. control.
† $P < 0.001$ vs. control.
‡ $P < 0.05$ vs. control.

mg/kg/day) of SPE in the bladder and only with a high dose (60 mg/kg/day) in the submaxillary gland. Similarly, a significant enhancement of B_{max} values for specific [^{3}H]prazosin binding was observed in the rat prostate by repeated treatment with the low dose (6 mg/kg/day) of SPE, but not in the submaxillary gland, spleen, and heart. On the other hand, in vitro experiment has shown that SPE exhibits little tissue selectivity in binding activities of each receptor. We have no clear explanation for such receptor binding selectivity by oral administration of SPE in the rat lower urinary tract. The most plausible reason may be preferential distribution of receptor binding constituents in the lower urinary tract after systemic administration of SPE. It is reported that SPE contains a complex mixture of free fatty acids and their esters, small quantities of phytosterols (eg, beta-sitosterol), aliphatic alcohols, and various polyprenic compounds.[20] A systemic distribution study in rats administered [^{14}C]oleic acid or [^{14}C]sitosterol-supplemented SPE has shown that these components are accumulated in the prostate to a greater extent compared with other tissues.[21] Because the prostate is particularly rich in free fatty acids, it would be expected that greater amounts of lipophilic substances accumulate in the prostate than in other tissues.

It is well known that chronic treatment with agonists and antagonists induces compensatory alteration of au-

tonomic receptor expression.[15,22,23] Saw palmetto extract has been shown to exert inhibitory effects against phenylephrine-induced formation of [^{3}H]inositol phosphate formation, which is suggestive of an alpha 1-adrenoceptor antagonistic effect.[11] Thus, it is plausible that a significant enhancement of [^{3}H]prazosin binding sites in the rat prostate after repeated administration of SPE reflects alpha 1-adrenoceptor upregulation due to the sustained receptor blockade. In fact, Kersting *et al.*[24] have shown that chronic treatment with prazosin may cause upregulation of alpha 1-adrenoceptors in patients with congestive heart failure. Furthermore, it is considered that a significant decrease of bladder [^{3}H]NMS binding sites in SPE-treated rats may represent downregulation of muscarinic receptors, resulting from the continuous receptor stimulation. Alternatively, there is a possibility that SPE contains slowly dissociating muscarinic antagonists, as previously suggested in the underlying mechanism for unsurmountable receptor antagonists.[25] It may be conceivable that apparent inverse compensation of autonomic receptors occurs, because SPE may be present as a mixture in agonists and antagonists for autonomic receptors.

CONCLUSIONS

The present study has revealed that SPE exerts significant binding activities on alpha 1-adrenergic and muscarinic receptors in the rat lower urinary tract. Thus, our data may contribute significantly to the further understanding of the pharmacologic effects of SPE in the treatment of patients with BPH and LUTS.

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【総　説】

ノコギリヤシ果実抽出液の排尿機能及び下部尿路受容体に対する作用

Effects of Saw Palmetto Extract on Urodynamic Function and Receptors in the Lower Urinary Tract

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【要　旨】
ノコギリヤシ果実抽出液 (SPE) は，ヨーロッパでは前立腺肥大症 (BPH) に対する治療薬として用いられ本邦でも健康食品として汎用されている．SPE の薬理作用には抗アンドロゲン作用などがある．臨床的には，SPE (320 mg/day) の 6 ヶ月投与により BPH やそれに伴う頻尿に有効との報告がある．排尿機能及び下部尿路受容体に対する SPE の作用を調べたところ，酢酸誘発頻尿ラットシストメトリーにおいて，SPE は排尿間隔及び一回排尿量を有意に増加させ，頻尿改善作用を示した．さらに SPE は前立腺 α_1 受容体，膀胱ムスカリン性及び 1,4-ジヒドロピリジン系 Ca 拮抗薬受容体に対し結合活性を示した．SPE は反復経口投与により，テストステロン誘発肥大前立腺における α_1 受容体数の増加を抑制した．また，SPE の反復投与はラットの血液臨床検査値，肝機能及び肝薬物代謝酵素活性に影響しなかった．以上，SPE は下部尿路受容体への直接作用による BPH の機能的閉塞の解除や頻尿の抑制などの薬理作用を示すことが示唆された．

【キーワード】
ノコギリヤシ果実抽出液，排尿パラメータ，前立腺 α_1 受容体，膀胱ムスカリン性受容体

1. はじめに

近年，わが国においては，代替医療の普及と高齢者人口の増加に伴い，健康増進や疾患の予防・治療を目的として健康食品への関心が高まっている．欧米では，民間薬として伝承されてきたメディカルハーブを医療の現場において積極的に活用しており，本邦でも健康食品として容易に入手可能である．特に高齢者では，医薬品とともに健康食品の摂取率が高く，この傾向は今後益々増加すると予想される．一方において健康食品の使用頻度が増大するに従い，その過剰摂取による健康被害や医薬品と併用した場合の有害事象が報告されている．しかしながら，メディカルハーブを含めいわゆる健康食品に関しては医薬品の場合と比較して，有効性についてそのメカニズムを含めた科学的検証は未だ十分とは言えず，健康食品それ自体の有害事象や医薬品との相互作用に関して信頼できる情報の提供も行われていない．従って，健康食品の適正な使用を確保するためには，有効性及び安全性に関する科学的検証が急務といえる．ここでは，近年特に注目されている健康食品のうち，前立腺肥大による排尿症状の改善目的で使用されるノコギリヤシ果実を取り上げ，それらの有効性及び安全性について，我々の最近の知見も交えて紹介する．

2. 前立腺肥大症とノコギリヤシ果実抽出液

食生活の欧米化や高齢者人口の増加に伴い，前立腺肥大症 (BPH) 患者が急増している．BPH は，前立腺組織の肥大による機械的閉塞や，前立腺平滑筋の α_1 受容体緊張亢進による機能的閉塞により排尿困難や残尿を呈する疾患である．この薬物治療には，現在 α_1 遮断薬やホルモン剤が汎用されるが，これらの医薬品では起立性低血圧や性機能障害などの副作用が問題となっており，また BPH 患者の約半数においては頻尿症状も出現することから，抗コリン薬も併用される場合も多い．カンザス大学の泌

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尿器外来に来院する男性患者を対象に代替医療食品の使用率を調査したところ，302 例中約半数の患者が前立腺の健康を目的にノコギリヤシを含めた何らかの代替医療食品を使用していた．これより，泌尿器系疾患の男性において，代替医療食品が普及していること，また年齢が上がるにつれその使用率も高まると報告している[1]．

ヤシ科シュロ属のノコギリヤシ果実抽出液 (saw palmetto extract: SPE) は，北米原産の低灌木で，南東部の海岸地帯の砂丘の松林に生息している．超臨界抽出法により精製されたイタリアインデナ社の SPE (SABALSELECT) は，飽和・不飽和脂肪酸が 90%以上を占め，その他，高級アルコール及びステロール等で構成されている．現在報告されている SPE の薬理作用としては，5α-reductase 阻害作用[2]，アンドロゲン受容体遮断作用[3]，抗炎症作用[4]，細胞増殖抑制作用[5]，α_1 受容体遮断作用[6]，鎮痙作用[7]などが知られている．しかし，SPE の有効成分が明らかになっていないこと，また本邦においてはいわゆる健康食品として扱われていることから，様々な規格の SPE が販売されている．そこで，SPE の全体像を明らかにする目的で，今まで報告されている SPE の臨床試験，また筆者らが実施した SPE の排尿機能に対する作用や排尿障害の薬物治療において標的部位となる下部尿路受容体（α_1 受容体やムスカリン性受容体など）に対する作用について紹介する．

3. ノコギリヤシ果実抽出液の臨床試験

SPE を用いた臨床試験を表 1 に示した[8-10]．これまでに 10 を超えるプラセボ対照試験[11-21] と 4 つの実薬対照試験[22-25] などが実施されている．

(1) プラセボ対照試験

プラセボ対照試験の SPE 投与量はいずれの試験においても 320 mg/日（160 mg/回，1 日 2 回）であった．このうち 1980 年代に報告された試験は，被験者数が限られており，また試験期間も短いものが多かった．一方，2000 年以降に発表された試験は，比較的被験者数も多く，試験期間も 6 ヶ月以上と長い．

多くの試験において，SPE の最大尿流量率及び夜間頻尿の改善が認められた．また，最近の試験で評価項目として用いられている IPSS（International Prostate Symptom Score：国際前立腺症状スコア）あるいは AUASI（American Urological Association Symptom Index：米国泌尿器科学会症状指標）では，2 つの試験で有効性が認められたものの，これらの指標が測定された他の 2 つの試験では認められなかった．結果のばらつきについては，被験者数，試験期間が各試験で異なっていること，さらに使用された SPE の規格が統一されていないこと，プラセボの識別不能性，対象患者の特性など，様々な要因が挙げられる[11]．今後，これらの点を考慮した試験の実施と結果の集積が望まれる．

(2) 実薬対照試験及び open 試験

SPE の実薬対照試験として，4 試験[22-25] が報告されている．

Grasso ら[24] は，SPE と Alfuzosin の比較試験の結果，Boyarsky's total score 及び排尿障害スコアにおいて Alfuzosin は SPE に比較して統計的に有意に優れていたことを報告している．一方，これ以外の実薬対照試験 3 試験[22,23,25] において SPE は既存の臨床薬とほぼ同様の効果を示した（表 1）．Alfuzosin を対照とした試験は，評価期間が 3 週間と短く，一方その他の試験は 3 ヶ月から 12 ヶ月と比較的長い．これまでに動物試験の結果から SPE の効果発現までには 2-3 ヶ月の期間が必要であることが示唆されており[32]，Alfuzosin を対照とした試験と他の 3 試験の結果が異なった一因として，試験期間の長短が関与しているものと考察される．

Debruyne ら[33] は，2002 年に報告した試験[22] をさらに層別解析し，重症 (IPSS>21) BPH 患者における刺激症状及び閉塞症状に対する SPE 及び Tamsulosin の効果を検討した結果，SPE は Tamsulosin に比較して同等以上の効果が認められたことを示した．しかしながらこの解析はレトロスペクティブなものであり，既存薬に対する SPE の効果についても更なる検証が必要である．現在，米国において National Institute of Diabetes and Digestive and Kidney Diseases（国立糖尿病・消化器・腎疾病研究所），National Center for Complementary and Alternative Medicine（国立補完代替医療センター），及び Office of Dietary Supplements をスポンサーとした前立腺肥大症に対する SPE, Pygeum africanum（アフリカ原産常緑樹であるピジウムの樹皮エキス），及び Tamsulosin を比較する大規模な第 III 相臨床試験が実施されている[34]．

4. ノコギリヤシ果実抽出液の排尿機能に対する作用

SPE の排尿機能に対する作用を検討するため，ウレタン麻酔下ラットを用いたシストメトリーにより膀胱内圧及び排尿量の測定を行った[35,36]．まず，生理食塩水を膀胱内に注入した正常ラットにおいて，SPE (6 mg/kg) を十二指腸内投与したところ，排尿間隔，一回排尿量，膀胱容量，静止時膀胱圧，排尿閾値圧及び最大膀胱収縮圧は投与前後で有意な差異がなく，排尿パラメータに殆ど影

表1 前立腺肥大症患者を対象とした臨床試験におけるSPEのIPSS，最大尿流量率及び夜間頻尿に及ぼす効果

Study	治療群	用量	試験期間	IPSS		最大尿流量率		夜間頻尿	
				n	change	n	change	n	change
プラセボ対照試験									
Bent et al., 2006	SPE	160*2	12 m	112	−0.68#	112	0.42		
	Placebo	Placebo		113	−0.72#	113	−0.01		
Willetts et al., 2003	SPE	160*2	12 m			46	1.5		
	Placebo	Placebo				47	4.4		
Gerber et al., 2001	SPE	160*2	6 m	41	−4.4	41	1.0		
	Placebo	Placebo		44	−2.2	44	1.4		
Marks et al., 2000	SPE (blend)	106*3	6 m	21	−2.24	21	1.27		
	Placebo	Placebo		23	−1.39	23	0.09		
Descotes et al., 1995	SPE	160*2	1 m			82	3.42	82	−0.67
	Placebo	Placebo				94	1.06	94	−0.32
Reece et al., 1986	SPE	160*2	3 m			33	2.35	33	−1.0
	Placebo	Placebo				37	2.3	37	−1.0
Cukier et al., 1985	SPE	2*80*2	2–3 m					43	−1.1
	Placebo	Placebo						47	−0.5
Tasca et al., 1985	SPE	160*2	3 m			14	3.3	14	−2.6
	Placebo	Placebo				13	0.6	13	−1.2
Champault et al., 1984	SPE	2*80*2	1 m			46	2.7	47	−1.4
	Placebo	Placebo				39	0.25	41	−0.5
Boccafoschi et al., 1983	SPE	160*2	2 m			11	4.13	11	−2.2
	Placebo	Placebo				11	1.96	11	−1.0
Emili et al., 1983	SPE	160*2	1 m			15	3.37	15	−1.6
	Placebo	Placebo				15	0.2	15	−0.4
実薬対照試験									
Debruyne et al., 2002	SPE	320*1	12 m	350	−4.4		1.79		
	Tamsulosin	0.4*1		354	−4.4		1.89		
Carraro et al., 1996	SPE	160*2	6 m	464	−5.8		2.68	464	−0.74
	Finasteride	5		477	−6.2		3.26	477	−0.69
Grasso et al., 1995	SPE	160*2	0.75 m			31	2.8	32	−1.0
	Alfuzosin	7.5				32	4.7	31	−0.9
Adriazola et al., 1992	SPE	160*2	3 m			20	1.5	20	−0.2
	Prazosin					22	0.47	22	−0.4
オープン試験									
Aliaev et al., 2002	SPE	160*2	60 m	26	−8.8		4.13		
Pytel et al., 2002	SPE	160*2	24 m	155	−5.33		1.13		
Stepanov et al., 1999	SPE	160*2	3 m	100	−6.5		1.4		
	SPE	320*1			−6.4		1.8		
Gerber et al., 1998	SPE	160*2	6 m	46	−7.0	46	−0.7		
Foroutan, 1997	SPE	2*80*2	3 m	592	−6.5	592	2.93	592	−1.0
Authie et al., 1987	SPE	2*80*2	3 m					500	−2.2

#: AUASI: American Urological Association Symptom Index

響しなかった（表2）．次に，高用量のSPE (60 mg/kg) 投与において，排尿間隔，一回排尿量及び膀胱容量はいずれも有意に（約40%）増加した（図1A, 表2）．次に，0.1%酢酸溶液をラット膀胱内に注入することにより，排尿間隔，一回排尿量及び膀胱容量は正常ラットの場合に比べ有意に減少し，頻尿状態が観察された．この頻尿モデルラットにSPE (12, 20 mg/kg) を投与することにより，排尿間隔，一回排尿量及び膀胱容量はいずれも投与量に依存して有意に増加し，頻尿改善作用を示すことが明らかになった（図1B, 表2）．このSPEの作用は正常ラットに比べ酢酸誘発頻尿ラットにおいて低用量で発現したことから，病態特異的である可能性が示された．ところで，

表2 麻酔下ラットシストメトリーにおけるSPE (6–60 mg/kg) 投与前後の排尿パラメータ

投与量 (mg/kg)	排尿間隔 (min)		一回排尿量 (mL)		膀胱容量 (mL)	
	投与前	投与後	投与前	投与後	投与前	投与後
正常ラット（生理食塩水注入）						
6	9.93±1.07	9.07±1.09	0.69±0.07	0.66±0.09	0.65±0.06	0.60±0.07
60	7.71±0.90	10.8±1.77*	0.54±0.06	0.77±0.13*	0.51±0.06	0.72±0.12*
頻尿ラット（0.1%酢酸溶液注入）						
12	6.44±1.10	7.41±1.25*	0.44±0.08	0.51±0.08*	0.43±0.07	0.49±0.08*
20	5.99±0.47	8.85±1.07*	0.40±0.04	0.66±0.08*	0.40±0.03	0.59±0.07*
投与量 (mg/kg)	静止時膀胱圧 (mmHg)		排尿閾値圧 (mmHg)		最大膀胱収縮圧 (mmHg)	
	投与前	投与後	投与前	投与後	投与前	投与後
正常ラット（生理食塩水注入）						
6	3.35±0.19	3.20±0.22	5.02±0.13	4.78±0.13	26.1±2.2	23.9±2.0
60	3.17±0.10	2.93±0.21	4.74±0.16	4.81±0.15	28.3±0.8	26.0±0.7*
頻尿ラット（0.1%酢酸溶液注入）						
12	3.99±0.26	3.69±0.23	5.05±0.31	5.13±0.27	29.3±1.7	25.7±2.0**
20	3.54±0.16	3.37±0.11*	4.44±0.19	4.62±0.25	25.6±1.4	23.0±0.5

*P<0.05, **P<0.01 vs. 投与前

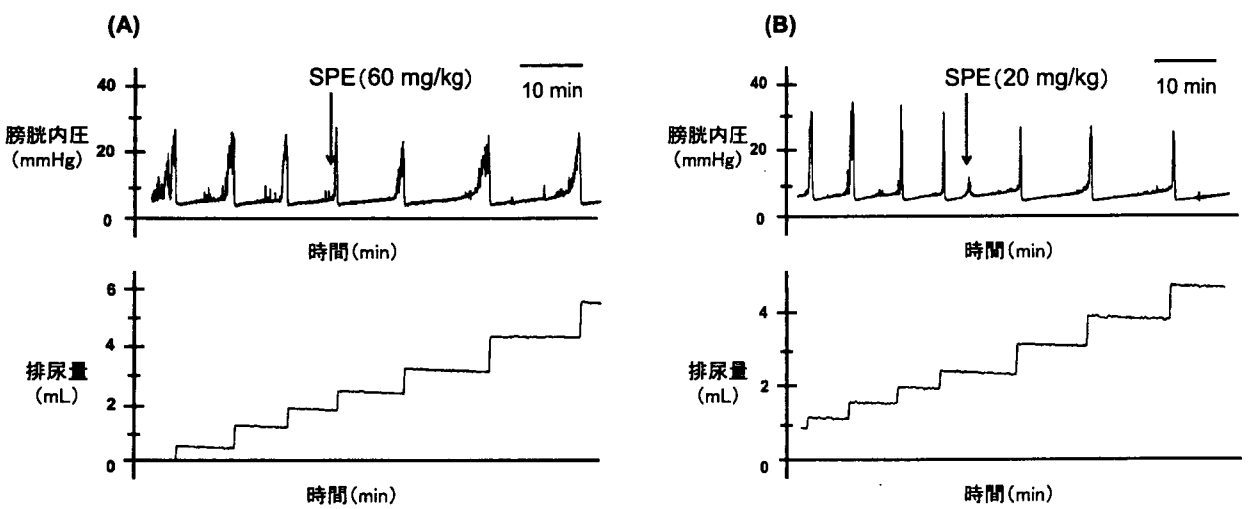

図1 麻酔下ラット（A: 生理食塩水注入，B: 0.1%酢酸溶液注入）シストメトリーにおけるSPE (A: 60 mg/kg, B: 20 mg/kg) 十二指腸内投与時のシストメトログラム．

SPEは高カリウム，ノルエピネフリン及びアセチルコリンによるラット摘出平滑筋組織の収縮を抑制する[7,37]ことから，*in vivo*において膀胱平滑筋の弛緩により頻尿改善作用を示すことが考えられた．また，SPEの主な含有成分である遊離脂肪酸は，K^+, Na^+, Ca^{2+}などのイオンチャネル透過性や神経伝達などの生理機能に対して影響を及ぼすこと[38]，並びに酢酸誘発頻尿は膀胱知覚神経の活性化に基づく[39]ことから，SPEは下部尿路神経に作用することも推定された．

5. *In vitro* におけるノコギリヤシ果実抽出液の各種受容体に対する作用

次に，排尿障害治療薬の作用部位となる下部尿路自律神経受容体（α_1 受容体，ムスカリン性受容体，1,4-ジヒドロピリジン (DHP) 系 Ca 拮抗薬受容体，ATP (P2X) 受容体）に対するSPEの結合活性を，各受容体の選択的標識リガンドを用いる受容体結合測定法により検討した[35,36]．その結果，SPEはラット前立腺及び脾臓の α_1 受容体（IC_{50} 値：それぞれ 169, 188 μg/mL），ラット膀胱及び顎下腺のムスカリン性受容体（IC_{50} 値：それぞれ 40.4,