Enhancement by Grapefruit Juice of Morphine Antinociception

Takashi Okura,[1] Tadahiro Ozawa, Yoshihiko Ito, Midori Kimura, Yoshiyuki Kagawa, and Shizuo Yamada*

Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) and Clinical Pharmaceutics, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan. Received July 8, 2008; accepted September 26, 2008; published online October 2, 2008

The aim of this study was to investigate the effect of grapefruit juice intake on the antinociception of morphine in rats. The antinociception of morphine (30 mg/kg, *per os* (*p.o.*)) was significantly enhanced by the oral administration of grapefruit juice (2 ml/rat). Further, the effect of grapefruit juice was examined in morphine-tolerant rats. The repeated administration of morphine (100 mg/kg *p.o.*) for 5 d caused a marked decrease in the antinociception, indicating the development of morphine-tolerance. In the morphine-tolerant rats, oral administration of grapefruit juice potentiated significantly the antinociceptive effect of morphine. To examine the pharmacokinetics of morphine after the repeated treatment with morphine for 5 d, microdialysis probes were implanted into the jugular vein and spinal intrathecal space in rats. The morphine concentrations in the blood and intrathecal cerebrospinal fluid (CSF) were gradually decreased by the repeated treatment with morphine. The grapefruit juice treatment significantly increased the blood concentration of morphine in morphine-tolerant rats. These results suggest that oral administration of grapefruit juice enhances the morphine antinociception by increasing the intestinal absorption of this agent.

Key words morphine; grapefruit juice; tolerance; microdialysis

Drug–food interactions are increasingly recognized as noteworthy clinical events that should be considered in order to avoid adverse effects. Indeed, the intake of grapefruit juice has been demonstrated to elevate serum concentrations of several drugs including calcium channel blockers such as felodipine, nifedipine and nisoldipine, verapamil, cyclosporine, tacrolimus and midazolam.[2—6] The main mechanism for the interaction with grapefruit juice is considered to be the inhibition of cytochrome P450 3A4 (CYP3A4), the major drug metabolism enzyme in the intestine. Recent investigations have shown that grapefruit juice inhibits not only CYP3A4 but also drug transporters like P-glycoprotein,[7—9] which plays important roles in the intestinal barrier function in a coordinated manner with CYP3A4.[10] The inhibitory effect of grapefruit juice on the intestinal barrier function may enhance the oral bioavailability of drugs, which has been associated with a higher incidence of side effects. From the point of view of beneficial use of dietary constituents, the enhancement of bioavailability can potentiate the therapeutic effect of drugs. Until now, few studies have focused on the beneficial use of interaction between dietary constituents and drugs.

Morphine is the most commonly used opioid analgesic for the treatment of cancer pain. Morphine is a substrate of P-glycoprotein,[11] and its antinociceptive effect is enhanced by knockout of the P-glycoprotein gene in mice and the administration of P-glycoprotein inhibitor in rats.[12—14] In humans, it has been reported that the absorption of morphine is regulated by intestinal P-glycoprotein.[15] Further, P-glycoprotein may be partially associated with morphine tolerance.[16] The tolerance limits the clinical use of morphine. We speculated that the intake of grapefruit juice inhibits the intestinal P-glycoprotein-mediated efflux of morphine and subsequently enhances the antinociceptive effect after an oral administration by increasing the drug's bioavailability.

In this study, we examined the effects of grapefruit juice intake on oral morphine antinociception in morphine-naïve and morphine-tolerant rats. The antinociceptive effect and concentrations of morphine in the blood and intrathecal cerebrospinal fluid (CSF) were monitored in rats treated repeatedly with morphine.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 250 g were housed three to four per cage with free access to food and water and maintained on a 12-h light/dark cycle in a room with controlled temperature (24±1 °C) and humidity (55±5%) throughout a whole experimental period. This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka. Morphine hydrochloride was purchased from Takeda Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were purchased from commercial sources.

Grapefruit juice (Sunkist®) was orally administered at a volume of 2 ml per rat 30 min prior to the drug administration in accordance with previous report.[17] In the single administration experiment, rats received 2 ml of water, grapefruit juice (Sunkist®) or quinidine (30 mg/kg *per os* (*p.o.*)), a P-glycoprotein inhibitor,[18] and 30 min later, morphine (30 mg/kg). The tail-flick latency test was used to quantify antinociception, with a thermal stimulus being applied to the tail.[19] Before the drug administration, baseline antinociceptive testing was performed. The antinociceptive testing was performed at 60, 120 and 180 min after the morphine treatment. A maximum tail-flick latency of 10 s was used to minimize the tissue damage to the tail. The tail-flick latency values were converted to a percentage of the maximum possible effect (%MPE): %MPE=(postdrug latency−predrug latency)/(maximum latency−predrug latency)×100.

In the repeated administration experiment, development of morphine tolerance was measured according to a method[20] described previously with a slight modification. Briefly, rats received morphine (100 mg/kg) orally once a day for 5 d, and

* To whom correspondence should be addressed. e-mail: yamada@ys7.u-shizuoka-ken.ac.jp

antinociception was determined by tail-flick test as described above once a day (120 min after morphine administration) to avoid tissue damage by repeated measurements. On the day (day 6) after the last treatment, 2 ml of grapefruit juice (Sunkist®) was orally administered to rats 30 min before another administration of morphine (100 mg/kg *p.o.*), and the tail-flick latency test was performed as described above.

The concentration of morphine in the blood and spinal CSF was determined by the microdialysis method. The spinal intrathecal dialysis probe was constructed from Cuprophan hollow fibers (inside diameter (i.d.), 0.2 mm; MW cut-off, 12500; RENAK-E, RE-10M, Kawasumi Chemical Industries Ltd., Tokyo, Japan).[21,22] The fibers were coated with epoxy glue, except for a 4-cm region in the middle. A Nichrome wire (outside diameter (o.d.), 0.1 mm; Unique Medical Co., Ltd., Tokyo, Japan) was then passed through the fiber and both ends of the fiber were attached to pieces of polyethylene tube (PE-10; Natsume Seisakusho Co., Ltd., Tokyo, Japan). Rats were anesthetized with ketamine (188 mg/kg intramuscular injection (i.m.)) and their heads were placed in a stereotaxic apparatus (SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan). The probe was inserted through an incision in the cisternal membrane and slowly passed caudally 9 cm into the intrathecal space to leave the uncoated section of the catheter at the Th11–L2 spinal segments. The two PE-10 ends of the dialysis probe were externalized on the top of the head. Rats were allowed to recover from the surgery for 3 d. They were then anesthetized with ether and a dialysis probe for vessels (TP-100-10, Eicom Corp., Tokyo, Japan) was implanted into the jugular vein. The dialysis probes implanted into the jugular vein and spinal intrathecal space were perfused at a constant rate of 5 μl/min with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$, pH 7.3) containing antipyrine as a reference of probe recovery.[23] After the oral administration of morphine (100 mg/kg) in rats, collection of the dialysate was started. The blood and spinal CSF dialysate samples were collected every 60 min for 300 min, and each sample was kept at −20 °C until the analysis. The dialysate concentration of morphine was measured by HPLC with fluorimetric detection.[24] The HPLC system consisted of a pump (880-PU, Japan Spectroscopic Co. (Jasco), Tokyo, Japan), a fluorescence detector (RF-535, Shimadzu, Tokyo, Japan) and an integrator (C-R6A, Shimadzu, Tokyo, Japan). The analytical column was composed of Nucleosil C18 ODS (4.6 mm×250 mm, 5 μm particle size, GL Sciences). Gradient elution was carried out at room temperature at a constant flow rate of 1.0 ml/min. Solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in 40% acetonitrile. The initial concentration of acetonitrile was 6.4%. After the injection of sample, the system was pumped isocratically for 2 min, followed by a gradient from 6.4 to 20% acetonitrile over 10 min, and then a gradient from 20 to 40% acetonitrile over 2 min to wash the column. The column elute was monitored fluorometrically at excitation and emission wavelengths of 280 and 335 nm, respectively. The concentration of morphine in blood (C_{blood}) or spinal CSF (C_{CSF}) was estimated from the dialysate concentration (C_d) using antipyrine as a reference.[23]

$$C_{blood} \text{ or } C_{CSF} = C_d/\{1-\exp(-R_{d,ref}PA_{vitro}/F)\}$$

F is the dialysate flow rate and PA_{vitro} is the *in vitro* permeability rate constant, which can be estimated from the *in vitro* recovery of the microdialysis probe.[23] $R_{d,ref}$ is the effective dialysis coefficient of the reference compound, antipyrine, which is the ratio of the *in vivo* and *in vitro* probe recovery.

The statistical analysis of the data was performed with Student's t-test for single comparisonss. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

In the single administration experiment, the morphine caused increases in latency in the tail-flick test in rats. The oral administration of grapefruit juice (2 ml/rat *p.o.* 30 min before the morphine administration) significantly increased the antinociception at 60 min after the morphine was administered (Fig. 1). The area under the effect–time curve (*AUE*) in grapefruit juice-treated rats was 1.5-times greater than that in control rats. The oral administration of quinidine, a P-glycoprotein inhibitor, increased markedly antinociception of morphine as shown by 2.8-fold greater *AUE* compared that in control rats (Fig. 2). On the other hand, the administration of grapefruit juice without morphine did not cause antinociceptive effects (data not shown). These results suggest that grapefruit juice enhances the antinociceptive effect of morphine in rats, though the antinociception increase by grapefruit juice was smaller than that by quinidine.

In the repeated administration experiment, the rats received morphine (100 mg/kg) orally once a day for 5 d. An antinociception was measured by the tail-flick test 120 min after receiving morphine. The antinociception was 100%

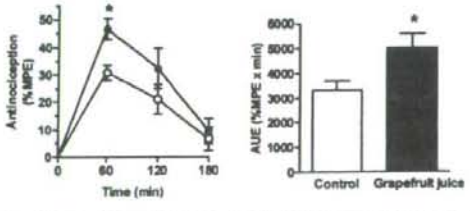

Fig. 1. Effects of Grapefruit Juice Administration on Antinociception of Morphine in Rats

Rats received water (control) (○) or grapefruit juice (2 ml/rat) (●) 30 min before morphine administration (30 mg/kg *p.o.*). The tail-flick test was conducted 60, 120 and 180 min after the morphine administration. The area under the effect–time curve (*AUE*) for antinociception of morphine was calculated by a trapezoidal role. Each point and column represents the mean±S.E. for five rats. * $p<0.05$ *vs.* control.

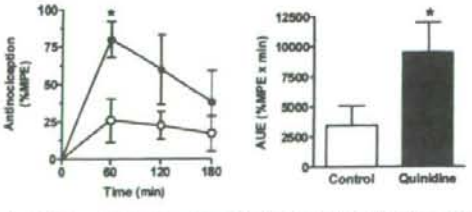

Fig. 2. Effects of Quinidine Administration on Antinociception of Morphine in Rats

Rats received water (control) (○) or quinidine (30 mg/kg *p.o.*) (●) 30 min before morphine administration (30 mg/kg *p.o.*). The tail-flick test was conducted 60, 120 and 180 min after the morphine administration. The area under the effect–time curve (*AUE*) for antinociception of morphine was calculated by a trapezoidal role. Each point and column represents the mean±S.E. for four rats. * $p<0.05$ *vs.* control.

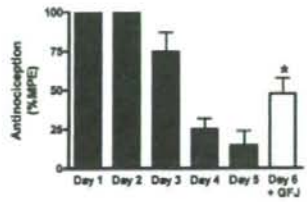

Fig. 3. Effects of Repeated Oral Administration of Morphine on the Antinociceptive Action of This Agent in Rats

Morphine (100 mg/kg) was given orally once a day for 1—5 d. After 120 min, rats were subjected to the tail-flick test. On the day (day 6) after the 5-d-treatment with morphine, rats received grapefruit juice (GFJ) (2 ml/rat) 30 min before receiving morphine. The data are presented as % MPE. Each column represents the mean±S.E. for four rats. * $p<0.05$ *vs.* day 5.

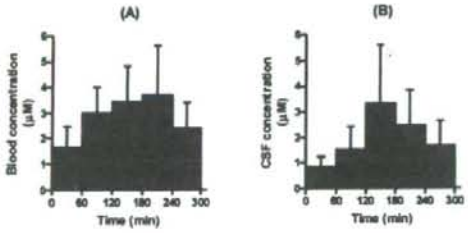

Fig. 4. The Concentration of Morphine in the Blood (AUC_{blood}) (A) and Spinal CSF (AUC_{CSF}) (B) of Morphine after Oral Administration on Day 1 in Rats

Microdialysis probes were implanted into the jugular vein and intrathecal space. Rats received orally morphine (100 mg/kg). After the morphine administration, dialysate samples were collected every 60 min for 300 min, and the concentrations were measured. Each column represents the mean±S.E. for four rats.

MPE on day 1 and 2, and 75, 25 and 14% MPE, respectively, on day 3, 4 and 5 (Fig. 3). Tolerance developed with the repeated oral administration of morphine. The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat *p.o.*) 30 min before receiving morphine. The pretreatment with grapefruit juice significantly enhanced the antinociception of morphine from 14% MPE (day 5) to 48% MPE.

To determine the pharmacokinetics of morphine during the development of morphine-tolerance, a microdialysis method was applied to the jugular vein and spinal intrathecal space. The concentrations of morphine in blood and spinal CSF on day 1 increased with time and reached maximum levels at 180—240 min in blood and 120—180 min in spinal CSF, respectively (Fig. 4). The concentrations of morphine in blood and spinal CSF gradually decreased during the oral treatment (Fig. 5). The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat *p.o.*) 30 min before receiving morphine. The AUC_{blood} was significantly (1.9 times) greater on day 6 than day 5 (Fig. 5A). The AUC_{CSF} was increased 1.3 times by the grapefruit juice, but not significantly (Fig. 5B). The concentration ratio of AUC_{CSF} to AUC_{blood} (AUC_{CSF}/AUC_{blood}) was 0.62, 0.45, 0.67 and 0.50 on day 1, 3, 5 and 6, respectively. The increases in the plasma concentration of morphine caused by grapefruit juice may contribute at least partly to the enhancement of morphine antinociception.

Grapefruit juice and its constituents are considered to affect the functions of drug transporters such as P-glycoprotein,[7—9] multidrug resistance protein 2 (MRP2)[25] and organic anion transporting polypeptide (OATP),[26] in addition to the drug metabolism enzyme CYP3A4. Of these enzymes and transporters, P-glycoprotein can affect morphine's disposition, because morphine is a substrate for P-glycoprotein, but not CYP3A4 or MRP2.[11—14] It was reported that the inhibition of intestinal P-glycoprotein by oral administration of quinidine enhances the absorption and pharmacological effect of morphine in humans.[15] Oral administration of quinidine also elevated the antinociception of orally-administered morphine in rats. Grapefruit juice extracts cause a four-fold increase in the transport of [^{3}H]vinblastine, a P-glycoprotein substrate, from apical to basolateral sides across human intestinal Caco-2 cells.[8] De Castro *et al.*[9] have reported that 6′,7′-epoxybergamottin, 6′,7′-dihydroxybergamottin, naringin and naringenin in grapefruit juice inhibit the P-glycoprotein-mediated transport of talinolol in human intestinal Caco-2 cells with IC_{50} values of 0.7, 34, 236 and 2409 μM, respectively. They have suggested that these furanocoumarins and flavonoids are able to inhibit intestinal P-glycoprotein-mediated transport because they are present in grapefruit juice in the same concentration ranges.[27,28] Taken together, oral administration of grapefruit juice may potentiate the antinociceptive effect of morphine by increasing the intestinal absorption possibly *via* the inhibition of intestinal P-glycoprotein.

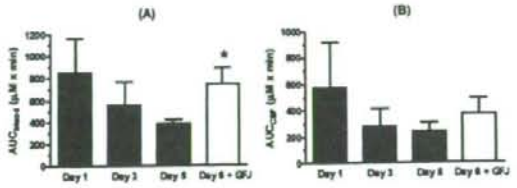

Fig. 5. Area under the Concentration Curve of Morphine in the Blood (AUC_{blood}) (A) and Spinal CSF (AUC_{CSF}) (B) of Morphine after Oral Administration for 1—5 d in Rats

Microdialysis probes were implanted into the jugular vein and intrathecal space. Rats received orally morphine (100 mg/kg) once a day. On the day (day 6) after the 5-d-treatment with morphine, rats received grapefruit juice (GFJ) (2 ml/rat) 30 min before receiving morphine. The concentrations of morphine in the dialysates were measured on day 1, 3, 5 and 6. Each column represents the mean±S.E. for three to four rats. * $p<0.05$ *vs.* day 5.

P-glycoprotein at the blood–brain barrier modulates the antinociceptive effect of morphine by regulating its transport from the blood into the central nervous system.[12—14] However, the spinal CSF to blood concentration ratio of morphine was not changed by grapefruit juice, suggesting little or insignificant inhibition of P-glycoprotein at the brain barrier. Although flavonoids and furanocoumarins are partially absorbed from the intestine, flavonoids such as naringin are most likely hydrolyzed by intestinal enzymes[29,30] and bergamottin has very low permeability through CYP3A4-expressing Caco-2 cell monolayers.[31] In addition, bergamottin and dihydroxybergamottin strongly bind to human serum albumin. These dispositional properties may explain in part why grapefruit juice inhibits intestinal CYP3A4 rather than hepatic CYP3A4 *in vivo*.[6] The unbound concentrations of these flavonoids and furanocoumarins in blood may be too low to inhibit P-glycoprotein at the luminal membrane of the blood–brain barrier.

Aquilante *et al.*[16] have reported that repeated morphine

administration causes a two-fold increase in the P-glycoprotein level in rat brain associated with the decrease in the antinociceptive effect. In morphine-tolerant rats, intestinal P-glycoprotein-mediated transport may be stimulated and therefore more susceptible to the inhibition of intestinal P-glycoprotein. Thus, inhibitors of intestinal P-glycoprotein such as grapefruit juice may partly overcome morphine-tolerance, though little clinical evidence has been presently reported on enhancement of effects of morphine by grapefruit juice. In fact, it may be difficult to control the intestinal P-glycoprotein activity using grapefruit juice, because the amounts of flavonoids and furanocoumarins may differ with area, season, and production process.[27,28] In addition, it has been suggested that grapefruit juice–drug interaction is caused by additive or synergistic effects of several flavonoids and furanocoumarins in the juice.[32] Thus, further quantitative analysis will be required to clarify the mechanism underlying enhancement of the antinociception of morphine by grapefruit juice.

In conclusion, grapefruit juice is suggested to potentiate the antinociception of morphine associated with an increase in intestinal absorption. This enhancement may partially overcome morphine-tolerance.

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Antinociceptive Effects of St. John's Wort, *Harpagophytum Procumbens* Extract and Grape Seed Proanthocyanidins Extract in Mice

Shinya UCHIDA,[a] Keita HIRAI,[a] Junya HATANAKA,[b] Junko HANATO,[a] Keizo UMEGAKI,[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] *Yokohama Oils and Fats Industry Corporation; 1–1 Minamiasama-cho, Nishi-ku, Yokohama 220–0074, Japan: and* [c] *National Institute of Health and Nutrition; 1–23–1 Toyama, Shinjuku-ku, Tokyo 162–8636, Japan.*

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Hypericum perforatum **extract (St. John's wort, SJW),** ***Harpagophytum procumbens*** **extract (HPE) and Grape seed proanthocyanidin extract (GSPE) have a broad spectrum of biological activities including antidepressant, anti-inflammatory or anti-oxidant effects. The aim of this study was to clarify antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. Also, the effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were examined. Oral pretreatment with SJW (100—1000 mg/kg) and HPE (30—300 mg/kg) attenuated significantly times of licking/biting both first and second phases of formalin injection in mice in the dose-dependent manner, and GSPE (10—300 mg/kg) suppressed second phase. Naloxone (5 mg/kg, s.c.) significantly attenuated antinociceptive effect of HPE but not SJW and GSPE. Formalin injection resulted in significant increase in the content of nitrites/nitrates (NO_x) in mouse spinal cord. The rise of spinal NO_x content by formalin was significantly attenuated by HPE and SJW. The pretreatment with SJW significantly potentiated an antinociceptive effect of morphine (0.3 mg/kg, s.c.), although concentrations of morphine in plasma and brain were not significantly changed by these herbal extracts. In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test of mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibly by pharmacodynamic interaction.**

Key words antinociceptive effect; St. John's wort; *Harpagophytum procumbens* extract; Grape seed proanthocyanidin extract; morphine; interaction

Currently, the consumption of dietary supplement containing botanical products and foods is growing at a remarkable speed, in terms of the promotion of health or prevention and treatment of diseases. The extract from *Hypericum perforatum* (St. John's wort, SJW) possess clinical efficacy in the therapy of mild to moderate depression.[1,2] The most important constituents of SJW are phloroglucinols such as hyperforin and pseudohyperforin, naphthodianthrones such as hypericin and pseudohypericin, in addition to flavonoids such as rutin, quercetin, quercitrin. Several *in vitro* studies have indicated that SJW and hyperforin may act *via* a blockade of reuptake of serotonin, noradrenaline and dopamine in similar manner as most of the current antidepressants such as tricyclic antidepressants,[3—5] which have been known to exhibit antinociceptive properties by monoamine reuptake blockade.

Harpagophytum procumbens extract (HPE) and Grape seed proanthocyanidins extract (GSPE) have been reported to exert anti-inflammatory activity in rodents.[6—8] *Harpagophytum procumbens* commonly known as Devil's claw is an herbaceous plant, growing specifically in Southern Africa. Preparations of its secondary roots contain iridoid glycosides, mainly harpagoside, harpagogide and procumbide. HPE have been shown to possess clinical efficacy in the treatment of degenerative rheumatoid arthritis, osteoarthritis and tendonitis.[6—8] In addition, experimental study has revealed anti-inflammatory activity of HPE in Freund's adjuvant-induced arthritis model.[9] Proanthocyanidins are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers and bark. Grape seed proanthocyanidins, a combination of biologically active polyphenolic flavonoids including oligomeric proanthocyanidins, have been shown to exert a novel spectrum of biological, pharmacological, therapeutic and chemoprotective properties against oxygen free radicals and oxidative stress. GSPE protects against free radicals models and has exhibited superior antioxidant performance as compared to vitamin C, E and β-carotene.[10]

Previous studies with antidepressant activity of SJW and anti-inflammatory effects of HPE and GSPE have led to the idea that these herbal products exert antinociceptive action. Thus, the aim of this study was to clarify the antinociceptive properties of SJW, HPE and GSPE after oral administration to mice with mechanisms that might potentially underlie these activities. The effects of these herbal extracts on the antinociception and plasma and brain concentrations of morphine were also examined.

MATERIALS AND METHODS

Drugs SJW and HPE were kindly donated by Indena (Milan, Italy). SJW was standardized to the content of hypericin (0.3%) and hyperforin (3.2%) and HPE was also standardized to the content of harpagoside (1.9%). GSPE was kindly supplied by Kikkoman Co. (Chiba, Japan), and standardized to the content of proanthocyanidin (83.9%). Morphine hydrochloride was purchased from Takeda Pharmaceutical Co. (Osaka, Japan). All other drugs and materials were obtained from commercial source. Morphine, naloxone and formalin were dissolved in 0.9% NaCl. SJW was suspended

* To whom correspondence should be addressed. e-mail: yamada@u-shizuoka-ken.ac.jp

in distilled water and sonicated for 10 min before oral administration. HPE and GSPE were dissolved in distilled water.

Animals Male ICR mice (Japan SLC Inc., Shizuoka, Japan) weighting 20—30 g were used. Animals were housed under a 12-h light/dark cycle in a room with controlled temperature (24±1 °C) and humidity (55±5%). They were allowed free access to food and water prior to the experiments. All animal procedures were in strict accordance with the guideline approved by the Experimental Animal Ethical Committee of University of Shizuoka.

Formalin Test In the formalin test, mice were adapted in open Plexiglas observation cambers at 1 h before injection of formalin. Formalin (20 μl of a 2.5% solution in saline) was injected subcutaneously into the dorsal surface of right hind paw of mice using a Hamilton microsyringe with a 30-gauge needle, as previously described.[11] Each mouse was immediately returned to the observation chamber after formalin injection. A mirror was placed behind the chamber to allow the unhindered observation of formalin-injection paw. The time spent for licking or biting of injected paw (nociceptive response) was measured with stopwatch at 5 min intervals until 40 min post formalin injection and considered as a quantitative indication of nociception. The sum of time of licking/biting from 0 to 5 min was considered as the first phase, whereas the second phase was taken as the sum of time for licking/biting from 10 to 30 min. SJW (100—1000 mg/kg), HPE (30—300 mg/kg), GSPE (10—300 mg/kg) or vehicle (control group) were orally administrated to different groups of mice 60 min before formalin injection. Naloxone (5 mg/kg), yohimbine (3 mg/kg) and methysergide (3 mg/kg) were subcutaneously administered just before oral administration of these herbal extracts and the formalin test was performed 60 min after the administration of herbal extracts.

To examine effects of these herbal extracts on the antinociception of morphine, animals received SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle at 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Formalin test was performed at 15 min after morphine administration.

Tail-Flick Test Tail flick latency was measured using the automated tail flick analgesia meter (MK-330B, Muromachi kikai, Tokyo, Japan), as previously described[12] with minor modification. A noxious beam of light was focused on the tail about 4 cm from the tip, and the tail flick latency was recorded automatically to the nearest 0.1 s. The intensity of the radiant heat source was adjusted to yield baseline latencies between 2 and 4 s. Each mouse was given one test to determined baseline latency to tail-flick with a cutoff of 10 s set to avoid tissue damage. Animals were administrated SJW (1000 mg/kg), HPE (1000 mg/kg), GSPE (1000 mg/kg) or zvehicle, and tail flick latencies were determined at 30, 60, 90, 120, 150 and 180 min after administration.

Measurement of Locomotor Activity The locomotor activity of mice was assessed in the open-field test as described previously.[13] Open field was a 30×30 cm acrylic area with 30 cm high black walls surrounding the field. Thin black stripes were painted across the floor dividing the field into 9 squares of equal area and the number of squares crossed with all paws crossing was counted in a 5-min session.

Determination for Contents of Nitrites/Nitrates (NO_x) in Brain and Spinal Cord SJW (1000 mg/kg), HPE (300 mg/kg), GSPE (300 mg/kg) or vehicle was administrated to mice orally, and thereafter, brain and spinal cord were dissected 80 min after the administration. Other groups of animals received herbal extracts at 60 min before formalin injection in the same way as formalin test, and brain and spinal cord were removed 20 min after formalin injection. The content of NO_x in mouse brain and spinal cord were determined as previously described with minor modification.[14] Brain and spinal cord of mice were homogenized in 3 volume distilled water and centrifuged at 15000 *g* for 20 min at 4 °C. Fifty microliters of supernatant of tissue homogenate was mixed with 20 μl of 0.31 M potassium phosphate buffer (pH 7.5), 10 μl of 0.86 mM β-nicotinamide adenine dinucleotide phosphate (β-NADPH), 10 μl of 0.11 mM flavin adenine dinucleotide (FAD) and 20 mU of nitrate reductase. Samples were incubated for 60 min at room temperature in the dark. Then, 5 μl of 1 M $ZnSO_4$ was added to the sample and centrifuged at 15000 *g* for 10 min 4 °C. One hundred microliters of Griess reagent (1 : 1 mixture of 1% sulphanilamide in 5% H_3PO_4 and 0.1% *N*-(1-naphytyl)ethylendiamine) was added to 80 μl of supernatant and the mixture incubated for 10 min at room temperature. Absorbance was measured at 550 nm by a micro plate reader (Perkin-Elmer Life Sciences) and converted to NO_x content by using a nitrate standard curve.

Measurement for Morphine Concentration in Plasma and Brain SJW (300 mg/kg), HPE (30 mg/kg), GSPE (30 mg/kg) or vehicle was orally administered to mice 45 min before the treatment with morphine (0.3 mg/kg, s.c.). Mice were sacrificed 30 min after morphine administration, and blood and brain were collected. Plasma was separated by centrifugation. Morphine concentrations in plasma and brain were determined by HPLC with electrochemical detector as previously described.[15] Plasma sample (200 μl) was mixed with 50 μl of 2 μM naloxone (internal standard) and 800 μl of 0.5 M ammonium sulfate (pH 9.3). Brain sample was homogenized in 4 volumes of saline. The homogenate (1 ml) was mixed with 50 μl of 2 μM naloxone and 100 μl of 1 M perchloric acid and centrifuged at 2000 *g* for 10 min at 4 °C. The supernatant was transferred to another tube containing 2 ml of 0.5 M ammonium sulfate (pH 9.3). The mixture from plasma or brain sample was then applied to the Oasis HLB cartridge (Waters, Milford, MA, U.S.A.), which was pretreated with 1 ml methanol and 1 ml distilled water. Morphine was eluted with 1 ml methanol after the cartridge was washed with 4 ml of 15% methanol in 5 mM ammonium sulfate (pH 9.3). The elute was evaporated under a stream of nitrogen at 40 °C. The residue was dissolved in 200 μl of the mobile phase and 50 μl of this solution was injected into HPLC system. The HPLC analysis was constructed with a pump (LC-20AD, Shimadzu, Kyoto, Japan), an electrochemical-detector (Coulochem III, ESA Inc., Chelmsford, MA, U.S.A.) and a injector (SIL-20AC, Shimadzu, Kyoto, Japan). The separation was performed on an analytical column (CAPCELLPAK SCX UG80, 5 μm, 100×3 mm, Shiseido, Tokyo, Japan). The mobile phase consisted of 67% acetonitrile and 33% 20 mM potassium dihydrogen phosphate (pH 2.1) at a flow rate of 0.5 ml/min. The HPLC column was maintained at 40 °C and the electrochemical detector was set to +250 mV for detector 1, +600 mV for detector 2 and 800 mV for the guard cell.

Statistical Analysis All values are expressed mean± S.E. Date were analyzed by Student's *t*-test or one-way analysis of variance followed by Dunnett's *post hoc* test. For all comparisons, differences were considered statistically significant at $p<0.05$.

RESULTS

Effects on Nociceptive Responses in the Formalin and Tai-Flick Test The s.c. injection of 2.5% formalin into the right hind paw of mice induced a biphasic licking/biting nociceptive response. SJW at doses of 500 and 1000 mg/kg reduced significantly the licking/biting time both first phase (20.0 and 24.9%, respectively) and second phase (37.2 and 56.5%, respectively) in the dose-dependent manner (Figs. 1a, b). Similarly, HPE at doses of 100 and 300 mg/kg also reduced significantly the licking/biting time in both first phase (18.1 and 27.1%, respectively) and second phase (42.5 and 59.0%, respectively) (Figs. 1c, d). GSPE at doses of 30, 100 and 300 mg/kg reduced significantly (31.7, 38.3 and 48.1%, respectively) the licking/biting time in the second phase but not in the first phase (Figs. 1e, f).

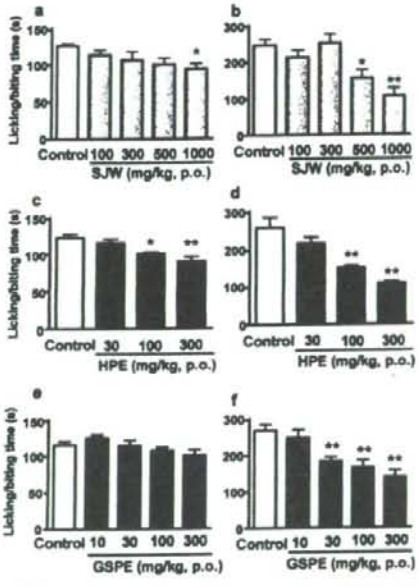

Fig. 1. Effects of SJW, HPE and GSPE on Licking and Biting Responses in First (a, c, e) and Second Phases (b, d, f) of Formalin Test in Mice

Mice received orally SJW (100, 300, 500, 1000 mg/kg) (a, b), HPE (30, 100, 300 mg/kg) (c, d) and GSPE (10, 30, 100, 300 mg/kg) (e, f) 60 min before the formalin injection to mouse paw. The licking/biting time (s) was measured. Each value represents mean±S.E. ($n=6$). Asterisks show a significant difference from control mice, $*p<0.05$, $**p<0.01$.

The effects of naloxone, yohimbine and methysergide on antinociception of SJW, HPE or GSPE in the formalin test were examined. Naloxone (5 mg/kg, s.c.) attenuated significantly (65.9%) antinociceptive effect in the second phase of formalin test by HPE (300 mg/kg, *p.o.*), but not by SJW (1000 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) (Fig. 2). None of these agents-induced antinociceptive responses in the first phase was attenuated by naloxone. In addition, naloxone at this dose effectively reversed morphine (0.3 mg/kg)-induced antinociceptive response both first and second phase. Yohimbine (3 mg/kg, s.c.) and methysergide (3 mg/kg, s.c.) did not significantly influence the antinociceptive effect of SJW (1000 mg/kg) both first and second phase (data not shown).

In the tail-flick test, there were little significant differences of tail-flick latencies between vehicle-treated group and each group treated with SJW (1000 mg/kg), HPE (1000 mg/kg) or GSPE (1000 mg/kg) (Fig. 3). Morphine increased significantly the latency at 30 and 60 min after administration.

Effects on the Locomotor Activity in Mice In open-field test, the numbers (counts/5 min) of crossing in mice 60 min after the pretreatment with vehicle, SJW (1000 mg/kg, *p.o.*), HPE (300 mg/kg, *p.o.*) and GSPE (300 mg/kg, *p.o.*) were 106±7, 118±8, 110±7 and 114±8, respectively. Thus, these herbal extracts had little significant effect on the locomotor activity.

Effects on the Contents of NO_x in Brain and Spinal Cord The formalin injection induced a significant (1.8 fold) incease of NO_x contents in mouse spinal cord but not in the brain. The formalin-induced increase of NO_x content in the spinal cord was significantly reversed by the pretreatment with SJW or HPE but not GSPE (Table 1).

Effects on the Antinociceptive Effect and Concentration in Plasma and Brain of Morphine Morphine at the dose of 0.3 mg/kg significantly reduced the licking/biting time in the first phase (24.8%) and the second phase (36.1%) of formalin test in mice. The antinociceptive effect (reduc-

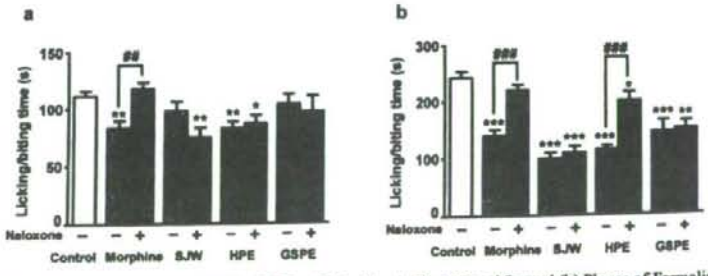

Fig. 2. Effect of Naloxone on Antinociception of SJW, HPE, GSPE and Morphine in First (a) and Second (b) Phases of Formalin Test in Mice

Mice received naloxone (5 mg/kg, s.c.) before oral administration of herbal extracts (SJW: 1000 mg/kg, HPE: 300 mg/kg, GSPE: 300 mg/kg) and then the formalin test was performed 60 min after the administration of herbal extracts. Naloxone was treated 45 min before the administration of morphine (0.3 mg/kg, s.c.), and the formalin test was performed 15 min after the administration of morphine. Each value represents mean±S.E. ($n=6$). Symbols show a significant difference from control mice (*,**,***) or from the corresponding mice without naloxone (#,##), $*p<0.05$, $**p<0.01$, $***p<0.001$, $\#p<0.01$, $\#\#p<0.001$.

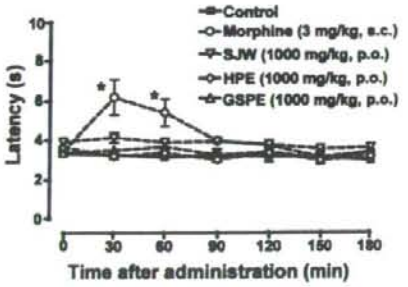

Fig. 3. Effects of SJW, HPE, GSPE and Morphine on Nociceptive Responses in Tail-Flick Test of Mice

Tail-flick latency was measured every 30 min after oral (s.c. for morphine) administration of each agent (SJW, 1000 mg/kg; HPE, 1000 mg/kg; GSPE, 1000 mg/kg; morphine, 3 mg/kg). Each value represents mean±S.E. (n=7—8). Asterisks show a significant difference from control mice at the same time, * p<0.05.

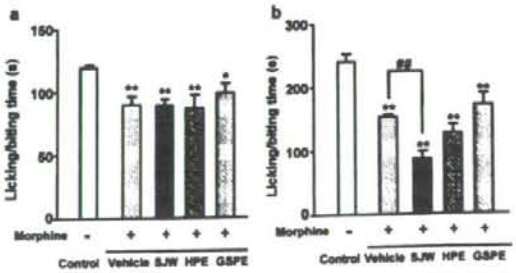

Fig. 4. Effects of SJW, HPE and GSPE on Antinociception of Morphinein in First (a) and Second (b) Phases of Formalin Test in Mice

Mice received orally herbal extracts (SJW, 300 mg/kg; HPE, 30 mg/kg; GSPE, 30 mg/kg) 45 min before morphine administration (0.3 mg/kg, s.c.). The formalin test was performed at 15 min after morphine administration. Each value represents mean±S.E. (n=6—7). Symbols show a significant difference from control mice (*,**) or from mice treated with morphine (#), * p<0.05, ** p<0.01, # p<0.01.

Table 1. Effects of Oral Administration of SJW, HPE and GSPE on Contents of NO_x in Mouse Brain and Spinal Cord

Treatment	Contents of NO_x	
	Brain (nmol/g)	Spinal cord (nmol/g)
Without formalin		
Vehicle	122.6±15.4	89.2±9.7
SJW	89.3±3.3	91.7±5.0
HPE	87.3±4.2	87.4±4.3
GSPE	113.2±20.4	120.0±7.5
With formalin		
Vehicle	130.9±7.3	161.7±11.5**
SJW	112.4±5.1	119.8±13.6#
HPE	121.1±7.8	116.0±9.9#
GSPE	106.6±4.7	152.1±8.3**

Formalin (2.5%, 20 μl) was injected 60 min after oral administration of SJW (1000 mg/kg), HPE (300 mg/kg) and GSPE (300 mg/kg). Brain and spinal cord were removed 20 min after formalin injection. Each value represents mean±S.E. (n=6—8). Symbols show a significant difference from vehicle group without formalin (**) or from vehicle group with formalin (#), ** p<0.01, # p<0.05.

Table 2. Effects of Oral Administration of SJW, HPE and GSPE on Plasma and Brain Concentration of Morphine in Mice

Treatment	Concentration of morphine	
	Plasma (ng/ml)	Brain (ng/g)
Vehicle	29.4±2.9	13.0±1.2
SJW	31.1±3.8	10.5±1.1
HPE	33.3±7.0	15.3±0.8
GSPE	33.8±5.0	13.5±1.9

Morphine (0.3 mg/kg, s.c.) was administrated to mice 45 min after oral administration of SJW (300 mg/kg), HPE (30 mg/kg) and GSPE (30 mg/kg). Blood and brain samples were collected at 30 min after morphine administration. Each value represents mean±S.E. (n=3—4).

tion of licking/biting time) of morphine in the second phase of formalin test was significantly potentiated by pretreatment with low dose (300 mg/kg) of SJW (Fig. 4). On the other hand, HPE (30 mg/kg) and GSPE (30 mg/kg) had little significant effect on the antinociceptive effect of morphine. The antinociceptive effect of morphine in the first phase was unaffected by these herbals.

The concentrations of morphine in plasma and brain 30 min after s.c. injection of morphie (0.3 mg/kg) in mice pretreated with SJW, HPE and GSPE were not significantly different from those in morphine-treated mice without these herbals (Table 2).

DISCUSSION

In the present study, we investigated antinociceptive properties of SJW, HPE and GSPE in mice with mechanisms that might potentially underlie these activities. SJW and HPE attnuated significantly nociceptive (licking/biting) responses in both first and second phase of formalin test. In contrast, GSPE was significantly efficacious only against the second phase. In the formalin test, it is considered that first phase of formalin-induced behavior reflects direct activation of A-delta and C afferent fibers while the second phase reflects both ongoing peripheral sensory input and central sensitization. Therefore, it is suggested that antinociceptive effects of SJW and HPE are mediated through central or both central and peripheral antinociceptive effects while the effect of GSPE is mainly due to the peripheral effect.

Antidepressant drugs such as tricyclics have been widely used in the treatment of patients with chronic pain. The serotoninergic and adrenergic neuronal systems in CNS may be significantly involved in the descending pain-inhibitory pathways,[16,17] and the interference of tricyclic antidepressant drugs with reuptake of serotonin and noradrenaline may be responsible for their antinociceptive activity.[18] There are accumulating evidences that antidepressant activity of SJW results from the suppression of reuptake of synaptic serotonin and noradrenaline.[3—5,19] Therefore, it is plausible that antinociceptive effect of SJW is attributable partly to the activation of descending serotoninergic and adrenergic pathways. However, in the current study, the antinociceptive effect of SJW was little affected by yohimbine (α_2 adrenoceptor antagonist) and methysergide (serotonin receptor antagonist). Chatterjee *et al.*[3] showed that hyperforin inhibited the uptake of GABA and L-glutamate, with similar IC_{50} values for the inhibition of uptake of serotonin, noradrenaline and dopamine. Thus, there is a possibility that the inhibition of GABA and L-glutamate uptake may be significantly associated with the antinociceptive effect of SJW. The antinociceptive effect of SJW was unaffected by naloxone, a non-specific antagonist of opioid receptors, suggesting that this ef-

fect of SJW is not mediated by opioid receptor system. Notably, relatively low dose of SJW significantly potentiated antinociceptive effect of morphine in the second phase of formalin test. It has been reported that tricyclic antidepressant drugs potentiate antinociceptive effect of morphine both animals and human and possess clinical efficacy in the treatment of chronic pain states such as an adjuvant analgesic.[18—22] Thus, it might be rational that SJW having antidepressant effect enhances antinociceptive effect of morphine.

Many botanical dietary supplements contain pharmacologically active phytochemicals that, when consumed concomitantly with conventional medications, may result in pharmacokinetic and/or pharmacodynamic interaction. SJW is a botanical supplement recognized for interacting with prescription medications.[23—25] SJW has been shown to decrease significantly blood concentrations of drugs such as indinavir, cyclosporine and midazolam by inducing particularly cytochrome P450 3A4 activity, thereby reducing the efficacy of drugs.[23—25] In the present study, there was no significant change in plasma and brain concentrations of morphine after oral administration of SJW, in spite that antinociceptive effect of morphine was effectively enhanced by pretreatment with SJW (Fig. 4). Therefore, this potentiation by SJW of morphine effect may be attributable to the pharmacodynamic interaction rather than pharmacokinetic interaction.

The antinociceptive effect of HPE in the formalin test (second phase) was significantly antagonized by naloxone, suggesting the involvement of opioidergic mechanism. There are three subtypes of opioid receptors (μ, δ, κ), and μ-opioid receptor activation is mainly involved in the antinociceptive effect of morphine. Our preliminary radioreceptor binding study has shown that HPE binds to μ-opioid receptors in mouse brain. Therefore, these results suggest a contribution of μ-opioid receptors in the antinociceptive effect of HPE.

Free radicals are implicated with pain[26,27] and some plant antioxidants have pain alleviating properties.[28] Proanthocyanidins are major polyphenols in grape seeds and they have potent antioxidant activities *in vitro* and *in vivo* models.[29—31] Since GSPE also contain high amount (84%) of proanthocyanidins, antioxidant activities of proanthocyanidins might contribute to the antinociceptive effect of GSPE. In fact, this notion is supported also by the observation that *Croton celtidifolius* extract containing high amount of proanthocyanidins (75%) exhibited antinociceptive effect in the formalin test.[32]

SJW, HPE and GSPE did not change latencies in the tail-flick test. It is considerd that tail-flick test is spinally mediated reflex to noxious stimuli. It is widely considered that analgesic effectiveness depended on the nociceptive stimulus, and that chemical stimulus (formalin test) is more sensitive than thermal one (tail-flick test).

There are considerable evidences to indicate that NO plays an important role in the processing of nociceptive transmission.[33,34] These include an increase in the release of excitatory neurotransmitter such as glutamate in the spinal cord following peripheral inflammation and an increase in the Ca^{2+} influx by glutamate resulting in the production of NO_x due to the activation of NO synthase. It was found that inhibition of NO synthase produced antinociception in the formalin test.[35—37] In this study, it was shown that formalin injection into one hind paw increased the content of NO_x in spinal cord, in accord with previously reports using microdialysis method.[38,39] Moreover, oral administration of SJW and HPE significantly inhibited the formalin evoked increase of NO_x content in the spinal cord. Thus, this result suggests that antinociceptive activity of SJW and HPE is related partly to the suppression of spinal NO pathway.

In conclusion, the present study has shown that SJW, HPE and GSPE exert significant antinociceptive effects in the formalin test in mice. In addition, opioidergic system seems to be involved in the antinociceptive effect of HPE but not SJW and GSPE. Furthermore, SJW potentiates morphine-induced antinociception possibility by pharmacodynamic interaction.

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Invited review

Pharmacological effects of saw palmetto extract in the lower urinary tract

Mayumi SUZUKI[1], Yoshihiko ITO[1], Tomomi FUJINO[1], Masasyuki ABE[1], Keizo UMEGAKI[2], Satomi ONOUE[1], Hiroshi NOGUCHI[1], Shizuo YAMADA[1]*

[1]*Department of Pharmacokinetics and Pharmacodynamics, Pharmacognosy and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka;* [2]*National Institute of Health and Nutrition, Tokyo, Japan*

Saw palmetto extract (SPE), an extract from the ripe berries of the American dwarf palm, has been widely used as a therapeutic remedy for urinary dysfunction due to benign prostatic hyperplasia (BPH) in Europe. Numerous mechanisms of action have been proposed for SPE, including the inhibition of 5α-reductase. Today, α_1-adrenoceptor antagonists and muscarinic cholinoceptor antagonists are commonly used in the treatment of men with voiding symptoms secondary to BPH. The improvement of voiding symptoms in patients taking SPE may arise from its binding to pharmacologically relevant receptors in the lower urinary tract, such as α_1-adrenoceptors, muscarinic cholinoceptors, 1,4-dihyropyridine receptors and vanilloid receptors. Furthermore, oral administration of SPE has been shown to attenuate the up-regulation of α_1-adrenoceptors in the rat prostate induced by testosterone. Thus, SPE at clinically relevant doses may exert a direct effect on the pharmacological receptors in the lower urinary tract, thereby improving urinary dysfunction in patients with BPH and an overactive bladder. SPE does not have interactions with co-administered drugs or serious adverse events in blood biochemical parameters, suggestive of its relative safety, even with long-term intake. Clinical trials (placebo-controlled and active-controlled trials) of SPE conducted in men with BPH were also reviewed. This review should contribute to the understanding of the pharmacological effects of SPE in the treatment of patients with BPH and LUTS.

Keywords: Saw palmetto extract, Pharmacological effects, Lower urinary tract receptors
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Introduction

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are very common disorders in aging men. The prevalence of histopathologic BPH is age dependent, with initial development usually occurring after 40 years of age[1]. By 60 years of age, its prevalence is greater than 50% and by age 85, the prevalence is as high as 90%. Similar to histological evidence, the prevalence of bothersome symptoms also increases with age. The two main forms of internationally accepted medical treatment for BPH are inhibitors of 5α-reductase, such as finasteride and α_1-adrenoceptor antagonists, with the latter being more effective[2]. In addition to these medications, the ripe berries of the American dwarf palm (Serenoa repens, saw palmetto) have been traditionally used to treat genitourinary problems; to enhance sperm production, breast size, or libido; and as a mild diuretic[3]. In many European countries, phytotherapeutic agents, including saw palmetto, are very popular. Phytotherapeutic agents represent nearly half of the medications dispensed for the treatment of BPH in Italy, compared with 5% for α-blockers and 5% for 5α-reductase inhibitors[4]. In Germany and Austria, phytotherapy is the first-line treatment for mild to moderate lower urinary tract symptoms and represents more than 90% of all drugs prescribed for the treatment of BPH[4–6]. Saw palmetto is a dwarf palm tree of the family Arecaceae and is indigenous to the southeastern parts of the United States. Saw palmetto berries have traditionally been used by American Indians to cure genitourinary disturbances, relieve mucous membrane irritations, increase testicular function, or increase breast size[5, 6]. In the United States, the use of phytotherapy for LUTS has grown rapidly, and approximately 2.5 million men use saw palmetto extract

* Correspondence to Shizuo YAMADA, Ph.D. Department of Pharmacokinetics and Pharmacodynamics and Global COE Program, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan.
E-mail: yamada@u-shizuoka-ken.ac.jp
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(SPE), although a guideline panel did not recommend phytotherapy as a treatment for BPH[7, 8]. In Japan, SPE is not a prescribed medication; however, it has been receiving increasing attention recently among patients with BPH.

The mechanisms of pharmacological action of SPE are not fully understood, although numerous proposals have been made, including inhibition of 5α-reductase, anti-androgenic effects, anti-proliferative effects, anti-inflammatory effects and anti-edema effects[6]. However, most of these pharmacological effects were observed at relatively high concentrations or large doses of SPE[9, 10], and it is uncertain whether the reported modes of action of SPE are therapeutically relevant[11, 12]. As described above, α_1-adrenoceptor antagonists are commonly used in the treatment of men with voiding symptoms (urinary obstruction, pollakiuria and urinary incontinence) secondary to BPH. Goepel *et al*[13] have shown that SPE may have α_1-adrenoceptor inhibitory properties. SPE significantly affects pharmacological receptors, such as the α_1-adrenoceptor and the muscarinic receptor in the lower urinary tract, to relieve the irritative and obstructive symptoms of dysuria due to BPH and LUTS[14]. In addition to traditionally used medications, like α_1-adrenoceptor antagonists, antimuscarinics, 5α-reductase inhibitors, and phytotherapy, several new therapeutic agents, such as selective β_3-adrenoceptor agonists, are potentially useful for treating LUTS suggestive of BPH, particularly for storage symptoms secondary to outflow obstruction[15]. Thus, the effects of SPE on these receptors in the lower urinary tract might be pharmacologically relevant.

To date, more than 11 placebo-controlled trials and 4 active-controlled trials with SPE in men with BPH have been conducted. Most of these were reported in the 1980s. Patient numbers were usually limited and the evaluation periods were relatively short, so it would be difficult to evaluate the effect of SPE and ascertain the efficacy of SPE in BPH patients. However, some placebo-controlled studies and comparisons to α_1-blockers have recently been conducted with relatively long-term treatments and sufficient numbers of patients[8, 16, 17].

Herbal products, including SPE, are often used with other prescription medications, and most patients with BPH are aged men. Elderly individuals frequently take dietary supplements with prescription drugs, and such a tendency will continue to increase in the near future. In such cases, a major concern is adverse events caused by a large excess intake or interactions between dietary supplements and drugs. Thus, the safety, as well as the efficacy, of these natural products and of their active ingredients remains to be analyzed at a scientific level. This review introduces newly revealed pharmacological actions of SPE, as well as some well-known mechanisms of action of SPE, and also summarizes clinical trials of SPE in comparison with currently used medicines.

Chemical composition

SABALSELECT™, manufactured by Indena S.p.A. (Milano, Italy), was used for the animal experiments[14, 18, 19]. Indena S.p.A. explains the extraction of saw palmetto in the brochure as follows: the fruits of *S repens* are extracted with supercritical CO_2. This extractive procedure, conducted at 45 °C/220 bar, directly produces a pharmacological product (SABALSELECT™), which can be used without further purification. Table 1 shows the chemical composition of SABALSELECT™. It consists of fatty acids, alcohols and sterols (Brochure of Sabalselect™: Indena S.p.A.). Habib and Wyllie[20] reported that the contents of different brands of SPE were markedly different; for example, free fatty acids ranged from 40.7 to 80.7% (mean %), methyl and ethyl esters from 1.5 to 16.7% (mean %), and glycerides from 6.8 to 52.2% (mean %). In the United States, herbal products are regulated under the Dietary Supplement Health and Education Act (DSHEA); however, approval for launching products onto the market is not required except in cases of a new dietary ingredient. Therefore, herbal products that existed before October 15, 1994, can remain with different ingredients[21]. Levin and Das[22] issued a warning that each

Table 1. Chemical Composition of SPE (Brochure of SabalselectTM: Indena S. p. A. http://www.indena.it/pdf/sabalselect.pdf).

Fatty acids	Content (%)	Fatty alcohols and sterols	Content (%)
Total fatty acids	93.5	Fatty alcohols	0.20
		Hexacosanol	0.017
Saturated	59.8	Octacosanol	0.146
Caproic acid	1.5	Tetracosanol	0.004
Caprylic acid	2.3	Triacontanol	0.003
Capric acid	2.5		
Lauric acid	30.2	Sterols	0.32
Myristic acid	12.0	Campesterol	0.07
Palmitic acid	9.5	Stigmasterol	0.03
Stearic acid	1.8	β-Sitosterol	0.22
Unsaturated	33.7		
Oleic acid	28.5		
Linoleic acid	4.6		
Linolenic acid	0.6		

*: Brochure of SabalselectTM: Indena S. p. A. http://www.indena.it/pdf/sabalselect.pdf

preparation must be considered individually because of differences in extraction techniques, preparation of products, composition, and biological activities.

Pharmacological properties

BPH causes dysuria and residual urine via a mechanical stoppage due to hypertrophy of prostatic tissue and via a functional stoppage caused by α_1-adrenoceptor hypertonia of prostatic smooth muscle. Previous studies have demonstrated that SPE has a number of pharmacological effects: 1) an anti-androgenic effect — inhibition of 5α-reductase I and II and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors, 2) an anti-inflammatory effect, 3) an anti-proliferative effect, (Figure 1), and 4) significant binding of pharmacological receptors existing in the lower urinary tract.

Anti-androgenic effects

The development and growth of the prostate gland depend on androgen stimulation[23, 24]. DHT is one of several factors regulating this development and growth[24, 25] and is converted from testosterone by 5α-reductase. This enzyme has two isoforms (5α-reductase 1 and 2)[25]. The respective roles of these 5α-reductases in BPH development have not yet been elucidated[26]. SPE inhibited both isozymes in a noncompetitive manner[27-29], whereas finasteride inhibited only 5α-reductase 2 in a competitive manner[25]. Among the many components of SPE, lauric acid and linoleic acid showed inhibition of both isozymes, oleic acid was active only against 5α-reductase 1 and myristic acid was active only against 5α-reductase 2. However, palmitic acid, stearic acid, esterified fatty acids, sterols, and alcohols were inactive against both[30].

Di Silverio *et al*[26] reported a significant decrease in DHT and increase in testosterone in the periurethral region of prostate tissue from BPH patients receiving Permixon® (320 mg/day) for 3 months and thus suggested that SPE could inhibit 5α-reductase in the human prostate *in vivo*. Sultan *et al*[9] investigated the interaction of SPE with the intercellular androgen-receptor complex. SPE inhibited [^{3}H]dihydrotestosterone from binding to its receptor. The affinity of SPE was higher for cytosol receptors than for nuclear receptors. Competitive interference with the binding of [^{3}H]methyltrienolone to cytosolic androgen receptors was also shown in rat prostate cells [31].

Anti-inflammatory effects

Inflammation was frequently observed in hormonally induced hypertrophied prostates of dogs[32] and in a study of human BPH[33]. Mahapokai *et al*[32] concluded that the development of hyperplasia preceded inflammatory infiltration. An anti-inflammatory effect was indicated as one of the mechanisms of action of SPE. In fact, it is plausible that SPE affects several inflammatory mediators. SPE showed anti-inflammatory and anti-edematous effects *in vivo*[34]. The production of 5-lipoxygenase metabolites was inhibited by SPE (Permixon®) at a concentration of 5 μg/mL[35]. Breu *et al*[34] demonstrated that acid lipophilic compounds of SPE inhibited the biosynthesis of cyclooxygenase and 5-lipoxygenase metabolites with the same intensity as SPE.

Vela Navarrete *et al*[36] conducted a multicenter open pilot clinical study to make a comparison between a control group and an SPE (Permixon®) group in BPH patients. After

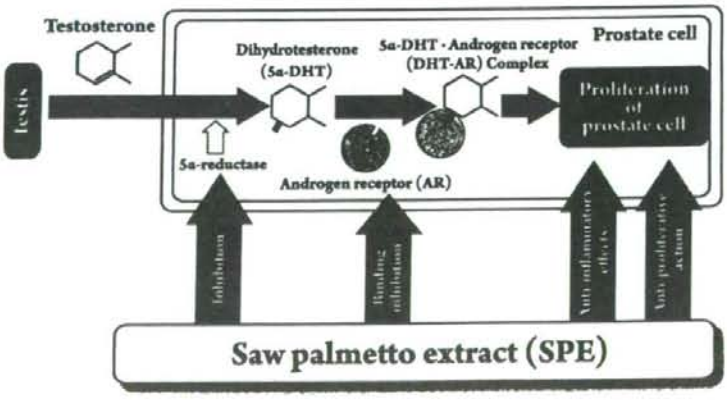

Figure 1. Mechanisms of pharmacological action of saw palmetto extract (SPE). They include antiandrogenic effects, such as inhibition of 5α-reductase I and II and inhibition of binding of dihydrotestosterone (DHT) to the cytosolic androgen receptors, anti-proliferative effects and anti-inflammatory effects.

3 months of treatment with SPE, the patients showed an improvement in their International Prostate Symptom Score (IPSS). Also, significant decreases in the levels of interleukin-1β (IL-1β) and tumor necrosis factor (TNF)-α were observed after the SPE treatment. Thus, SPE was shown to exert an anti-inflammatory effect.

Anti-proliferative effects

Maintenance of a constant number of cells is one of the basic functions of homeostasis. In normal adult prostate, the delicate balance between apoptosis and proliferation is well regulated and these indices are low. In contrast, in a prostate with BPH this equilibrium may not be maintained[37-40]. Kyprianou *et al*[37] showed a statistically significant elevation in TGF-β, a negative growth factor able to induce apoptosis under physiological conditions, in the epithelial cells of BPH tissue compared with the normal prostate and a statistically significant increase in the intensity of immunoreactivity for bcl-2 and the number of positive epithelial cells in BPH specimens relative to normal prostate. Claus *et al*[41] also indicated stromal growth in BPH due to cell proliferation in the absence of apoptosis. Vacherot *et al*[40] revealed that proliferation exceeded apoptosis in the stroma and epithelium of human BPH tissues. Although the rate of apoptosis did not differ between normal prostate and BPH tissue, the proliferative index was significantly higher in BPH tissue than in normal prostate in both the stroma and the epithelium. Furthermore, comparisons of the proliferative indices and apoptotic indices between the BPH tissues after 3 months of SPE (Permixon®) administration and those without SPE administration showed that in both the stroma and the epithelium, the proliferative index showed a significant decrease in SPE-treated BPH tissue relative to untreated tissue and the apoptotic index showed a significant increase in the SPE-treated BPH tissue.

Vacher *et al*[42] showed that SPE reduced the basal activity of K^+channels and of protein kinase C in Chinese hamster ovary cells and that pretreatment with SPE abolished the effects of prolactin. Furthermore, it was demonstrated that SPE (Permixon®) inhibited the effects of prolactin and androgens on prostate growth in the rat lateral prostate[23]. Thus, SPE might block prolactin-induced prostate growth by inhibiting several steps of prolactin receptor signal transduction.

Effects on pharmacological receptors in the lower urinary tract

In vitro effects

Goepel *et al*[13] have shown that SPE displaced an α_1-adrenoceptor radioligand to bind to human prostatic and cloned human α_1-adrenoceptors in a noncompetitive manner and concomitantly suppressed the agonist-induced formation of [^{3}H]-inositol phosphate. We evaluated the in vitro and in vivo binding of SPE to autonomic receptors in the lower urinary tract[14, 18, 19]. The in vitro experiment has shown that SPE inhibited the specific binding of [^{3}H]prasozin (α_1-adreceptor), [^{3}H]N-methylscopolamine (muscarinic receptor) and (+)-[^{3}H]PN 200-110 (1,4-dihydropyridine receptors), but not [^{3}H]αβ-MeATP (purinergic receptor), in the prostate, bladder and other tissues of rats in a concentration-dependent manner. Our recent study has shown that SPE competitively inhibited specific binding of [^{3}H]prasozin and [^{3}H]N-methylscopolamine in human prostate and bladder (Yamada et al., unpublished data). Thus, it is suggested that SPE binds to α_1-adrenergic, muscarinic and 1,4-dihydropyridine receptors, but not to purinergic receptors[14, 18, 19]. Based on IC_{50} values (Table 2), the binding activity of SPE for muscarinic receptors was shown to be 2-4 times greater than that for α_1-adrenergic and 1,4-dihydropyridine receptors. The affinity of SPE for these receptors was comparable to the in vitro pharmacological potency of this extract (*eg*, inhibition of 5α-reductase (IC_{50}: 71 μg/mL), anti-inflammatory effect (IC_{50} of cyclooxygenase and 5-lipoxygenase: 28.1 and 18.0 μg/mL, respectively) and anti-androgenic effect (IC_{50}: 1004 μg/mL))[34, 43] reported previously. Furthermore, Scatchard analysis has revealed that SPE caused a significant decrease in the maximal number of binding sites (Bmax values) of [^{3}H]prazosin, [^{3}H]NMS and (+)-[^{3}H]PN 200-110 in the prostate or bladder of rats (45%, 45% and 33%, respectively)[18, 19]. Therefore, it could be presumed that SPE binds non-competitively to α_1-adrenergic, muscarinic and 1,4-dihydropyridine receptors in rat tissues. Such insurmountable antagonism by SPE was previously

Table 2. IC_{50} values for in vitro inhibition by SPE of specific binding of [^{3}H]prazosin, [^{3}H]NMS, and (+)-[^{3}H]PN 200–110 in rat tissues.

Radioligands	IC_{50} values (μg/mL) (Mean±SE, n=4–9)
Specific [^{3}H]prazosin binding	
Prostate	169±24
Spleen	188±47
Specific [^{3}H]NMS binding	
Bladder	40.0±4.1
Submaxillary gland	52.3±4.4
Specific (+)-[3H]PN 200-110 binding	
Bladder	97.3 ±17.1

noted in human prostatic and cloned α_1-adrenoceptors[13].

Vanilloids exert their activity through the transient receptor potential vanilloid subtype 1 (TRPV1), a nonselective cation channel. TRPV1 has been shown to be located in urinary bladder epithelial cells[44]. The urothelial TRPV1 may play a role in concert with TRPV1 nerve fibers[45]. Thus, TRPV1 may play a significant role in the pathophysiology of bladder disease. Our recent study has also shown that SPE significantly inhibits the capsaicin-induced Ca^{2+} influx in HEK293VR11 cells expressing TRPV1 receptors[46]. Furthermore, SPE inhibited specific binding of [^{3}H]resiniferatoxin in HEK293VR11 cells in a concentration-dependent manner. Thus, it is assumed that SPE inhibits the activation of TRPV1 in the bladder.

***In vivo* effects**

Suzuki *et al*[18, 19] examined the effects of oral administration of SPE on autonomic receptors in rats. Repeated oral administration of SPE (SABALSELECT™) for 4 weeks produced a significant decrease of muscarinic receptor (specific [^{3}H]NMS binding) sites in the rat bladder and submaxillary gland[18, 19]. Notably, such a reduction in the number of [^{3}H]NMS binding sites was observed at relatively low doses (0.6, 6 mg·kg^{-1}·day^{-1}) of SPE in the bladder and only at a high dose (60 mg·kg^{-1}·day^{-1}) in the submaxillary gland[19]. On the other hand, a significant enhancement of α_1-adrenoceptor (specific [^{3}H]prazosin binding) sites was observed in rat prostate after repeated treatment with the low dose (6 mg·kg^{-1}·day^{-1}) of SPE, but not in the submaxillary gland, spleen and heart. The in vitro experiment showed that SPE exhibited little tissue selectivity in the binding of each receptor. These data suggest that SPE administered orally specifically affects muscarinic and α_1-adrenoceptors in the lower urinary tract. Although there is no clear explanation for such selectivity, the most plausible reason may be the preferential distribution of receptor-binding constituents in the lower urinary tract after the systemic administration of SPE. SPE contains a complex mixture of free fatty acids and their esters, small quantities of phytosterols (*eg*, β-sitosterol), aliphatic alcohols and various polyprenic compounds[47]. A systemic distribution study in rats administered [^{14}C]oleic acid or [^{14}C]sitosterol-supplemented SPE has shown that these components are accumulated to a greater extent in the prostate than in other tissues[48]. 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.

Repeated administration of SPE (100, 320 mg/kg) for 30 days inhibited prostatic hyperplasia induced by sulpiride in rats[23] and repeated administration of SPE (50 mg/kg) for 60 days also inhibited prostate hyperplasia induced by testosterone[10]. Our previous study has shown that repeated treatment with testosterone for 4 weeks resulted in significantly increased (1.7–1.8 times) prostate weight in rats [18]. However, repeated oral administration of SPE (6, 60 mg·kg^{-1}·day^{-1}) failed to significantly decrease tissue weight in any region of hypertrophied prostates of rats induced by the testosterone treatment. The reason why our data could not reproduce previous results might be the lower dosage and shorter treatment period. In agreement with the observation by Suzuki *et al*[18], Rhodes *et al*[49] noted that even high doses (180, 1800 mg/day) of SPE had no effect on prostatic hyperplasia in rats induced by testosterone treatment. The dosages (6 or 60 mg/kg) were comparable (320 mg/day) or 10 times higher than the dosage used for the treatment for BPH in humans.

Repeated treatment with testosterone in rats brought about a significant (62%) increase in prostatic α_1-adrenoceptor receptor sites. Such enhancement of prostatic α_1-adrenoceptor density in testosterone-treated rats was alleviated by the concomitant administration of SPE (SABALSELECT™, 6 mg/kg) [18]. Thus, oral administration of SPE has been suggested to attenuate up-regulation of α_1-adrenoceptors in rat prostate induced by testosterone. It may be concluded that SPE at a clinically relevant dose exerts a direct effect on the pharmacological receptors in the lower urinary tract, thereby improving urinary dysfunction in patients with BPH and overactive bladders.

Effects on hepatic drug-metabolizing enzymes and blood biochemical values

Although the usage of medical herbs has grown quickly as a complementary and alternative medicine, 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[50–53]. The proposed interactions would affect the pharmacokinetics and pharmacodynamics of drugs: absorption in the small intestine, metabolism in the intestine and liver, distribution to target organs, transport across cell membranes, and binding to specific receptors. Among these interactions, induction and inhibition of hepatic drug-metabolizing enzymes by herbal medicines or dietary compounds have been investigated. Suzuki *et al*[18] have shown that repeated oral administration of SPE in rats had little significant influence on the content and activities of hepatic drug-metabolizing enzymes. Markowitz *et al*[54] reported that SPE (320 mg/day for 14 days) for the treat-

ment of lower urinary tract symptoms suggestive of BPH did not alter plasma concentrations of probe drugs for cytochrome P-450 (CYP)2D6 and CYP3A4 activity in normal volunteers. Therefore, it is unlikely that SPE at generally recommended doses alters the disposition of co-administered drugs. Also, repeated oral administration of SPE in rats had little effect on blood biochemical parameters, except for a slight increase in the albumin value, suggestive of relative safety even with long-term intake[18].

Clinical trials

Clinical trials conducted with SPE in men with BPH are summarized in Table 3[55-57]. There have been more than 11 placebo-controlled trials[8, 17, 58-66] and 4 active-controlled trials[11, 15, 67, 68].

Placebo-controlled trials

As shown in Table 3, all placebo-controlled trials were conducted with SPE (320 mg/day) and placebo. Most of them were reported in the 1980s; the patient number was usually limited and the evaluation period was relatively short. More recently, two new and relatively large-scale placebo-controlled trials were conducted. One was reported by Willetts *et al*[17] and the other by Bent *et al*[8]. A double-blind placebo-controlled trial was held in Australia from January 1999 to March 2000[17]. One hundred men with symptomatic BPH, aged <80 years with a maximal urinary flow rate of 5–15 mL/s, were included

Table 3. Effect of SPE on IPSS, peak urinary flow rate (Q_{max}) and mean values of urinary frequency (nocturia) in men with BPH in clinical trials.

Study	Group	Dose	Duration	IPSS		Q_{max}		Nocturia	
				n	change	*n*	change	*n*	change
Placebo-controlled study									
Bent S *et al* [8]	SPE	160*2	12m	112	-0.68#	112	0.42		
	Placebo	Placebo		113	-0.72#	113	-0.01		
Willetts KE *et al* [17]	SPE	160*2	12m			46	1.5		
	Placebo	Placebo				47	4.4		
Gerber GS *et al* [58]	SPE	160*2	6m	41	-4.4	41	1.0		
	Placebo	Placebo		44	-2.2	44	1.4		
Marks LS *et al* [59]	SPE (blend)	106*3	6m	21	-2.24	21	1.27		
	Placebo	Placebo		23	-1.39	23	0.09		
Descotes JL *et al* [60]	SPE	160*2	1m			82	3.42	82	-0.67
	Placebo	Placebo				94	1.06	94	-0.32
Reece SH *et al* [61]	SPE	160*2	3m			33	2.35	33	-1.0
	Placebo	Placebo				37	2.3	37	-1.0
Cukier J *et al* [62]	SPE	2*80*2	2–3m					43	-1.1
	Placebo	Placebo						47	-0.5
Tasca A *et al* [63]	SPE	160*2	3m			14	3.3	14	-2.6
	Placebo	Placebo				13	0.6	13	-1.2
Champault G *et al* [64]	SPE	2*80*2	1m			46	2.7	47	-1.4
	Placebo	Placebo				39	0.25	41	-0.5
Boccafoschi C *et al* [65]	SPE	160*2	2m			11	4.13	11	-2.2
	Placebo	Placebo				11	1.96	11	-1.0
Emili E *et al* [66]	SPE	160*2	1m			15	3.37	15	-1.6
	Placebo	Placebo				15	0.2	15	-0.4
Active-controlled study									
Debruyne F *et al* [16]	SPE	320*1	12m	350	-4.4		1.79		
	Tamsulosin	0.4*1		354	-4.4		1.89		
Carraro JC *et al* [11]	SPE	160*2	6m	464	-5.8		2.68	464	-0.74
	Finasteride	5		477	-6.2		3.26	477	-0.69
Grasso M *et al* [67]	SPE	160*2	0.75m			31	2.8	32	-1.0
	Alfuzosin	7.5				32	4.7	31	-0.9
Adriazora SM *et al* [68]	SPE	160*2	3m			20	1.5	20	-0.2
	Prazosin					22	0.47	22	-0.4

#: AUASI: American Urological Association Symptom Index

in the trial and were randomized to a group receiving SPE (160 mg twice a day) or placebo. The treatment period was 12 weeks. The primary outcomes were changes in IPSS, maximal urinary flow rate, and the Rosen International Index of Erectile Function (IIEF). The IPSS score decreased over time in both treatment groups; however, there was no significant difference after 12 weeks of treatment between the groups. There were no significant differences between the two treatment groups in the quality of life (QOL) score, the maximal urinary flow rate, and the IIEF score.. On the other hand, each treatment group showed a significant improvement between week 0 and week 12. This trial was double-blind placebo-controlled, with high compliance and a low withdrawal rate; therefore, it could be regarded as a well-controlled trial. However, some of the results were unexpected, especially for the IPSS score and urine flow rates. The authors considered that it might be ascribable to a low IPSS at baseline, a small number of patients, and a relatively short trial period.

The other clinical trial was held in the United States from July 2001 to May 2004[8]. It was a double-blind placebo-controlled trial lasting 14 months (2 months screening, 12 months treatment). Two hundred twenty-five men aged >49 years, with a maximum urinary flow rate of <15 mL/s, were randomly assigned to receive SPE (160 mg twice a day) or placebo. The primary outcomes were changes in the American Urological Association Symptom Index (AUASI) and the maximal urinary flow rate. Secondary outcomes were changes in prostate size, residual urinary volume after voiding, QOL, laboratory values, and the rate of reported adverse effects[8]. No significant differences between the SPE and placebo groups were observed in the change in AUASI scores (mean difference: 0.04 point), maximal urinary flow rate (mean difference: 0.43 mL/s), prostate size, residual volume after voiding, QOL or serum prostate-specific antigen (PSA) levels during the one-year trial. The incidence of side effects was similar in the two groups. During the single-blind, placebo run-in period, there was a small but significant decrease in the AUASI score. Bent *et al*[8] considered the discrepancy between their results and results from previous trials and questioned the adequacy of blinding, whether certain attributes of participants were taken into account, and specification of the SPE preparations of the previous trials.

Active-controlled trials

Four active-controlled trials have been conducted with SPE in men with BPH (Table 3). Just as the placebo-controlled trials, half of the trials enrolled very limited numbers of patients and had very short evaluation periods. Two active-controlled trials recruited enough patients and had relatively long treatment periods (6 and 12 months).

One of these studies was a 6-month, double-blind, randomized trial that compared the effects of SPE (160 mg twice daily, Permixon®) with that of a 5α-reductase inhibitor (5 mg finasteride) in 1,098 men with moderate BPH using IPSS as the primary outcome[11]. Both SPE and finasteride decreased the IPSS (-37% and -39%, respectively), improved QOL (by 38 and 41%) and increased peak urinary flow rate (+25% and +30%). Prostate volume (-18%) and serum PSA levels (-41%) were markedly decreased by finasteride. On the other hand, SPE improved symptoms with little effect on prostate volume and no change in PSA levels. SPE fared better than finasteride in a sexual function questionnaire and resulted in fewer complaints of decreased libido and impotence. Both treatments relieved the symptoms of BPH in about two thirds of the patients but, unlike finasteride, SPE had little effect on so-called androgen-dependent parameters. This suggests that other pathways are also involved in the symptomatology of BPH.

The other trial was a comparison of SPE (Permixon®) with tamsulosin[16]. Eight hundred eleven men with symptomatic BPH were recruited and 704 patients were randomized to receive either tamsulosin (0.4 mg/d) or SPE (320 mg/d). At 12 months, IPSS decreased by 4.4% in each group and no differences were observed in either irritative or obstructive symptom improvements. The increase in maximal urinary flow rate was similar in both treatment groups. The mean prostate volume decreased by 0.99 mL in the SPE group, whereas it increased by 0.22 mL in the tamsulosin group. Prostate-specific antigen (PSA) remained stable, whereas prostate volume decreased slightly in SPE-treated patients. The tamsulosin group showed no significant changes in total PSA. The two compounds were well tolerated; however, evacuation disorders occurred more frequently in the tamsulosin group. This trial demonstrated that SPE and tamsulosin were equivalent in the medical treatment of lower urinary tract symptoms in men with BPH during and up to 12 months of therapy.

Debruyne *et al*[69] conducted a subset analysis of the trial mentioned above. One hundred twenty-four patients with severe LUTS (IPSS>19) were stratified: 59 and 65 patients had been randomized to the tamsulosin and SPE groups, respectively. At 12 months, total IPSS decreased by 7.8% with SPE and 5.8% with tamsulosin; the irritative symptoms improved significantly more with SPE. The superiority of SPE in reducing irritative symptoms appeared only 3 months into treatment and was maintained up to month 12. Further analyses were conducted with the most severely symptomatic patients. In this subgroup, the between-group difference was maximal as soon as month 3 and was maintained up to

month 12 for both irritative and obstructive IPSS. For the irritative symptoms, the difference between groups was statistically significant over this period. Although the number of patients decreased, the between-group difference was still statistically significant over this period for the irritative symptoms.

Adverse effects of SPE are rare and usually mild. They include constipation, decreased libido, diarrhea, headache, hypertension, nausea, urine retention and pancreatitis[3, 70]. In all randomized clinical trials in the meta-analysis[3], withdrawal rates (a rough indicator of patient acceptance) were 9.1% for SPE, 11.2% for finasteride and 7.0% for placebo. No herb-drug interactions have been described[54, 71]. However, in high throughput screening, SPE showed potent inhibition of the metabolic activity of CYP3A4, 2D6, and 2C9[72].

Conclusions

BPH and associated LUTS are common disorders in aging men. Plant extracts are widely used in the treatment of BPH and related LUTS. In fact, SPE has been widely used as a therapeutic remedy for BPH in Europe. In the United States and Japan, SPE is not a prescribed medication; however, it has received attention from patients with BPH.

It is suggested that SPE has various pharmacological mechanisms (*eg*, inhibition of 5α-reductase, anti-androgenic effects, anti-proliferative effects, anti-inflammatory effects and anti-edema effects). In addition, SPE may have α_1-adrenoceptor inhibitory properties. In addition to the α_1-adrenoceptor binding, we found significant binding to the muscarinic and 1,4-dihydropyridine receptors as novel mechanisms of pharmacological action of SPE in the lower urinary tract (Figure 2). Also, there is a possibility that SPE affects vanilloid receptor activity in the bladder. Anticholinergic agents are widely used for the treatment of OAB; therefore, inhibition of muscarinic receptors could be a novel pharmacological effect of SPE on the lower urinary tract for relief of irritative and obstructive symptoms of dysuria in BPH and LUTS. It is unlikely that the usefulness of SPE is limited by notable interactions with coadministered drugs or serious adverse events. Thus, this review may significantly contribute to the further understanding of the pharmacological effects of SPE in the treatment of patients with BPH and LUTS.

The constituents of different preparations of SPE differed markedly. The efficacy of SPE likely depends on the ingredients. Hence, it would be ideal to identify the active ingredients and to establish the optimal preparation in terms of efficacy and safety, or it should be recognized that the efficacy and the safety of SPE could differ according to brand.

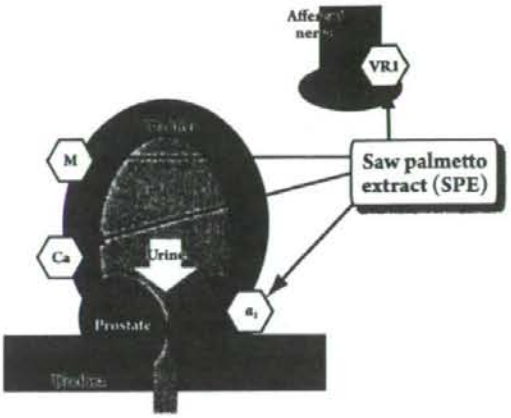

Figure 2. Proposed binding activities of saw palmetto extract (SPE) for pharmacological receptors in the lower urinary tract (bladder and prostate). M: muscarinic receptor, VR1: vanilloid receptor, Ca: 1,4-dihydropyridine receptor, α_1: α_1-adrenoceptor.

Considering that recent clinical trials, which were relatively large and well-controlled, did not demonstrate the superiority of SPE to placebo, the clinical potency of SPE has been questioned. However, the facts that several clinical studies showed the superiority of SPE over placebo and its comparability to prescribed medications and that many patients appear to reap benefits from SPE should be considered. Hence, it is anticipated that some suitably designed clinical studies (adequacy of blinding, treatment period, patient numbers, patient characteristics, etc.) will be conducted and we could ascertain the real potential of SPE for patients with BPH.

Abbreviations

SPE: saw palmetto extract
BPH: benign prostatic hyperplasia
LUTS: lower urinary tract symptoms
DSHEA: Dietary Supplement Health and Education Act
DHT: dihydrotestosterone
IPSS: International Prostate Symptom Score
TRPV1: transient receptor potential vanilloid subtype 1
CYP: cytochrome P-450
IIEF: International Index of Erectile Function
QOL: quality of life
AUASI: American Urological Association Symptom Index
PSA: prostate-specific antigen

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