SPECIAL PROCESSED PANAX GINSENG (SPPG) RELAXES ISOLATED RABBIT CORPUS CAVERNOSUM THROUGH HISTAMINE ANTAGONIZED PROPERTY AND WITH A BENEFICIAL EFFECT IN RAISING INTRACAVERNOUS PRESSURE

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The in vitro and in vivo effects of special processed Panax ginseng (SPPG) in rabbit corpus cavernosum were investigated. SPPG induced a concentration-dependent relaxation in phenylephrine-precontracted cavernosal strips with an EC50 of 0.39 ± 0.06 mg/ml and turned out to be more potent than total ginsenosides (1.33 ± 0.09 mg/ml). Comparison the HPLC profile of SPPG with 7 major ginsenoside standards indicated that Rg₁, Rf and Rc, with a percentage of 65, 20, and 2.5%, respectively, were the predominant component and precluding the involvement of Rb₁, Rb₂, Rd, and Re. The corporal relaxation of SPPG may be mediated by component(s) in addition to Rg₁/Rf/Rc because a reconstituted mixture did not mimic the potent relaxant effect of SPPG. Neither endothelial removal nor L-NAME treatment affect SPPG-induced relaxation. Histamine (10⁻⁸-10⁻⁴ M) produced concentration-dependent contraction of cavernosal strips. The contractile response to histamine was progressively suppressed both by H₁-receptor antagonist triprolidine and by SPPG (1, 2 and 4 mg/ml). On the other hand, SPPG-induced relaxation of phenylephrine-precontracted cavernosal strips was not attenuated by H₂ receptor antagonist cimetidine. Intracavernous (IC) injection of SPPG (0.5, 1, 2 and 4 mg/ml) to anesthetized rabbits, rose the IC pressure from basal (13.7 ± 4.2 mmHg) to 19.2 ± 5.4, 34.6 ± 4.2, and 46.7 ± 8.2 mmHg, respectively and prolonged the duration of tumescence ranged from 20 to 90 min. These finding indicate that non-major ginsenosides contribute to the beneficial corporal relaxant effect of SPPG, which is attributable to an endothelium-independent properties possibly link to antagonizing the H₁ receptor in the cavernosal smooth muscle. Furthermore, the in vivo effects of SPPG may implicate a potential for the treatment of erectile dysfunction.

Key words: Special processed Panax ginseng (SPPG), Corpus cavernosum, Intracavernous pressure, Histamine antagonist.
INTRODUCTION

Ginseng has been regarded as an aphrodisiac in the Orient from the ancient past, without any reported complications. There has been a clinical study on the effects of the consumption of ginseng on patients with erectile dysfunction. Previous studies also revealed that crude extract of ginseng or total ginsenosides exerts a direct relaxing effect on rabbit corpus cavernosal tissue in a dose dependent manner.

Relaxation of arterioles and arteries, increased intracavernous pressure and the reduction of venous outflow are key factors in the process of penile erection. Histamine is a potent bioactive amine closely involved in the regulation of vascular tone. In penile physiology, histamine has been suggested to be one of the neurotransmitters involved in penile erection. Kim et al. reported that H₁ receptor of histamine appears to be the principal functional histamine receptor subtype that mediates smooth muscle contraction in the corpus cavernosum and suggested that histamine H₁ receptor antagonists may have a role in future intra-cavernosal pharmacotherapy for erectile dysfunction. On the other hand, Cara et al. (1995) reported that histamine causes a dose-dependent relaxation of human corpus cavernosal strips which is significantly inhibited by the histamine H₂ receptor antagonist, cimetidine. Take together, the results of these studies point to the existence of contractile H₁ receptors and relaxant H₂ receptors in regulating penile smooth muscle tone.

The corporal relaxant effect of ginsenosides always linked to endothelium-dependent signal pathway. Ginsenosides however, is composed of a mixture of triterpene glycosides that may have complex mechanism(s), different or even counteracting effects. In this study, we prepared a special processed *Panax ginseng* (SPPG) and reported here that SPPG induces penile tumescence *in vivo* and exhibits more potent corporal relaxing effect than total ginsenosides by a mechanism mediated by antagonizing histamine H₁ receptor.

MATERIALS AND METHODS

HPLC analysis of ginsenosides composition in SPPG

Ginsenosides or SPPG were dissolved in methanol and were subjected to HPLC analysis. The HPLC system consisted of a Hitachi L-7100 pump, a Rheodyne Model 7725I injector and a Hitachi Model L-7450A photodiode-array detector. We followed a direct and rapid method for determining ginsenosides in ginseng crude extracts within 45 min as described by Chuang and Sheu. The separations were obtained by linear gradient elution, using eluents A and B [A: 10 mM KH₂PO₄−CH₃CN (80:20); B: H₂O−CH₃CN (15:85)] according to the following profile: 0-15 min, 98-96% A, 2-4% B; 15-25 min, 96-85% A, 4-15% B; 25-45 min, 85-75% A, 15-25% B; 45-61 min, 75-0% A, 25-100% B; 62-75 min, 100% B. The flow-rate was kept constant at 1.0 ml min⁻¹ and the peaks were monitored at 203 nm. The column used is Cosmosil 5C₁₈-MS (4.6 x 250 mm) (Hitachi, Japan). Twenty µl of sample solution was
injected into the HPLC system. Ginsenosides contained in sample were identified by comparing the retention times of authentic standards.

**In vitro organ bath experiments**

The corpus cavernosum was excised from adult (4-5 month) male New Zealand white rabbits (3.0-3.5 kg) under anesthesia with sodium pentobarbital and immediately placed in Krebs solution (mM: NaCl, 118; NaHCO₃, 25; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.2; glucose 10; CaCl₂ 2.5, pH 7.4). The corpus cavernosum was carefully dissected free from the surrounding tunica albuginea and mounted in organ baths maintained at 37°C (aerated with 5% CO₂, 95% O₂). Cavernosal strip was stretched to a resting force of 0.6-0.8 g and was equilibrated for at least 60 min. During this period, the tissue was washed with fresh solution every 15 min, and tension was adjusted if necessary. Some of the strips were exposed for 45 min to 0.5% CHAPS (wt/vol) to induce endothelial impairment and repeatedly rinsed with CHAPS-free media (10 times, every 5 min). All of the tissues treated with CHAPS did not relax or relaxed poorly (< 10% of maximal relaxation) to acetylcholine (1 µM) and were considered to be functionally denuded of endothelium. All experiments were performed in the presence of 3x10⁻⁶ M tetradotoxin and 10⁻⁵ M indomethacin to preclude the pre-ganglionic effect and the involvement of prostaglandin.

**Animal preparation for recording intracavernous pressure (ICP)**

All experimental protocols used in the present study received prior approval of the Animal Care and Use Committee of National Research Institute of Chinese Medicine. Male New Zealand white rabbits weighing 2.5-3.2 kg were used for this investigation. After sedation with an intramuscular injection of ketamine (10 mg/kg), the rabbits were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg). Anesthesia was maintained with 10 mg/kg as needed. The animals breathed spontaneously. The rabbits were then placed in the supine position, and the body temperature was maintained at 37°C using a heating pad and lamp. The femoral artery on one side was cannulated for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR) via a Gould 23 ID pressure transducer on Gould RS3400 polygraph. Under sterile conditions, the skin overlying the penis was incised and the corpora cavernosum was exposed at the root of the penis. A 25-gauge needle was inserted into the corpus cavernosum for pressure recording (Gould Polygraph RS3400). The needle was connected to a three-way stopcock, thus permitting the intracavernous injection of drugs. Tube was filled with heparinized saline (50 IU/ml) to prevent clotting.

In eight rabbits, increasing concentrations of SPPG were injected intracavernously in a consistent volume of 0.2 ml. Normal saline (NS, 0.2 ml) was injected in four rabbits as a control group. The effects of SPPG and NS on the ICP and the duration of action were evaluated. In order to minimize the effect of the previous drug, the
cavernous body was flushed with 0.2 ml NS before each injection and the time interval between each injection was at least 1 h.

**SPPG and other chemicals**

SPPG was specially prepared by Dr. Chou in our institute. Briefly, pulverized ginseng was extracted by refluxing with 60% ethanol (x 4) for 15 min at 60°C. After removal of the solvent by evaporation in vacuo, the EtOH extract was dissolved in water. This solution was partitioned into ether and aqueous layer, and the aqueous layer was further extracted with n-butanol (n-BuOH) saturated with water. The n-BuOH layer was then concentrated in vacuo and lyophilized to afford total ginsenosides. Fractionation of total ginsenosides was done by column chromatography on silica gel (Diaion HP20, 70-230 mesh, Nacalai Chemical Company) and eluted by a gradient of CH₂Cl₂/MeOH (from 10:0 to 0:10) mixture into 9 fractions (F1-F9). Each fraction was dried under reduced pressure and the corporal relaxant activity was further evaluated. In our preliminary study, F2 and F3 displayed more potent corporal relaxing effect than others. Thus, these two like fractions were combined and defined as SPPG.

It was first dissolved in distilled water and then serially diluted in normal saline (NS) immediately before experiments. Stock solution was used within 1 week after preparation. Acetylcholine hydrochloride, cimetidine, indomethacin, ketamine hydrochloride, L-N^G^-nitro arginine methyl ester (L-NAME), phenylephrine hydrochloride and tetradox toxin, were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Triprolidine hydrochloride was obtained from Research Biochemicals International (Natick, MA, USA). Ginsenosides Rb₁, Rb₂, Rc, Rd, Re, Rf and Rg₁ were purchased from Extrasynthese (Genay, France).

**Analysis of data**

Results were expressed as percentage of relaxation (%) running from 0% to 100% and used in the construction of the concentration-response curves. The EC₅₀ (mg/ml concentration required to cause half-maximal relaxation) was determined by liner interpolation for each concentration-response curve. The results are expressed as mean ± s.e.m. N designates the number of rabbits and n the number of corpus cavernosal strips examined in each experiment. Student’s t test (two-tailed) for paired or unpaired observations were used to determine the significance of difference for means. P values of less than 0.05 were considered to be statistically significant.

**RESULTS**

**HPLC chromatograms of ginsenosides in SPPG**

Ginsenosides contained in SPPG were identified by comparison the retention times with seven authentic standards (Rb₁, Rb₂, Rc, Rd, Re, Rf, and Rg₁). HPLC analysis showed that SPPG contains three predominant
ginsenosides. In terms of the individual constituents, Rg₁ (retention time = 19.53 min) existed in the highest content of 65%. Rf (retention time = 35.83 min) was also found about 20%. A trace component of Rc (~2.5%) was indicated as arrow (retention time = 41.46 min) (Fig. 1). Rb₁, Rb₂, Rd, and Re were precluded in SPPG. This experiment was repeated 3 times and obtained the same result.

Effects of SPPG on the corpus cavernosal strips

SPPG produced a concentration-dependent relaxation in phenylephrine-precontracted corpus cavernosum that was more potent than total ginsenosides (Fig. 2). According to the HPLC profile, SPPG contained about 65%, 20% and 2.5% of Rg₁, Rf, and Rc, respectively. Thus, we also evaluated the corporal relaxant effect of a Rg₁/Rf/Rc mixture. However, the Rg₁/Rf/Rc mixture at a reconstituted ratio of 26:8:1 did not mimic the corporal relaxant effect of SPPG.

![HPLC chromatogram of special processed Panax ginseng.](image)

**Fig. 1.** HPLC chromatogram of special processed *Panax ginseng*.

![Concentration-relaxation curves of total ginsenosides ( ), special processed *Panax ginseng* (SPPG, ) and a reconstituted mixture of Rg₁/Rf/Rc (26:8:1) ( ) in isolated rabbit corpus cavernosum precontracted with phenylephrine. Results are expressed as percentage of relaxation (%). Each point represents the mean±s.e.m. (n = 5-8).](image)

**Fig. 2.** Concentration-relaxation curves of total ginsenosides ( ), special processed *Panax ginseng* (SPPG, ), and a reconstituted mixture of Rg₁/Rf/Rc (26:8:1) ( ) in isolated rabbit corpus cavernosum precontracted with phenylephrine. Results are expressed as percentage of relaxation (%). Each point represents the mean±s.e.m. (n = 5-8).

![Concentration-relaxation curves of special processed *Panax ginseng* (SPPG) in phenylephrine-precontracted corpus cavernosum before ( ), after removal of endothelium ( ), and after Nω-L-nitroarginine methyl ester (10⁻³ M, ) treatment, respectively. Results are expressed as percentage of relaxation (%). Each point represents the mean±s.e.m. (n = 4-7).](image)

**Fig. 3.** Concentration-relaxation curves of special processed *Panax ginseng* (SPPG) in phenylephrine-precontracted corpus cavernosum before ( ), after removal of endothelium ( ), and after Nω-L-nitroarginine methyl ester (10⁻³ M, ) treatment, respectively. Results are expressed as percentage of relaxation (%). Each point represents the mean±s.e.m. (n = 4-7).
Effects of endothelium integrity on SPPG-induced relaxation

To evaluate endothelium-dependency, corpus cavernosal strips with or without endothelium were contracted with phenylephrine to similar magnitude and then exposed to SPPG. As shown in Fig. 3, removal of endothelium did not reduce SPPG-induced relaxation. Consequently, N\textsuperscript{G}-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, was employed to examine the possible involvement of nitric oxide (NO). Addition of L–NAME almost abolished 10\textsuperscript{-5} M ACh-induced maximal relaxation (< 7%), but failed to attenuate SPPG-induced response when compared with control groups.

Effects of SPPG on vasoconstrictors-evoked response

Cumulative addition of phenylephrine (PE) to the cavernosal strips evoked a concentration-dependent contraction. This contractile response was affected slightly by 1 mg/ml SPPG pretreatment (Fig. 4A). However, no further inhibition was observed when the concentration of SPPG was increased to 4 mg/ml. The effect of SPPG on KCl-induced contraction (Fig. 4B) was similar to that of phenylephrine.

Repeated, reproducible contractions of corpus cavernosum were also evoked by histamine (from 10\textsuperscript{-8} to 10\textsuperscript{-4} M). Triprolidine, a selective H\textsubscript{1} receptor antagonist, caused a suppression of the contractile response to histamine in a concentration-dependent manner (Fig. 5A), while H\textsubscript{2} receptor antagonist cimetidine had no inhibitory effect on it (data not shown). We further examined the effect of SPPG on histamine-induced contractile response. As shown in Fig. 5B, SPPG progressively attenuated histamine evoked corporal contraction with a pattern similar to triprolidine. Alternatively, the effect of SPPG on histamine-induced contraction was significantly distinguished from the effect on phenylephrine- and KCl-evoked contraction. On the other hand, triprolidine when added cumulatively induced a concentration-dependent relaxation in phenylephrine-precontracted corpus cavernosum.

![Fig. 4. Effects of special processed Panax ginseng (SPPG) pretreatment on phenylephrine (A) and on KCl (B) evoked corporal contraction. Results are expressed as percentage of vasoconstrictor-induced maximal contraction (%). Each point represents the mean±s.e.m. (n = 6-8). (○: control, □: 1 mg/ml SPPG, △: 2 mg/ml SPPG).](image-url)
Furthermore, pretreatment with cimetidine did not attenuate SPPG-induced corporal relaxation of PE-precontracted preparations, EC\textsubscript{50} values were 0.24 ± 0.08 and 0.33 ± 0.07 mg/ml, before and after cimetidine, respectively.

**In vivo effects of SPPG on ICP**

The baseline ICP recorded was 13.7 ± 4.2 mm Hg (Fig. 6A). Although transient rises or an unstable ICP following needle insertion was found occasionally, the ICP restabilized within 10-20 min. In all rabbits studied, intrapenile injection of SPPG induced tumescence as documented by a sustained increase in ICP. Fig. 6A shows the time-course changes in ICP after intracavernous injection 1 and 4 mg/kg of SPPG, respectively. During the injection periods, the MAP and HR were not changed significantly (81 ± 6 vs. 77 ± 4 mmHg and 213 ±17 vs. 211 ± 9 beats/min, respectively; before and after SPPG injection). Intracavernous injection of normal saline (NS) induced a transient rise in ICP, nevertheless, the pressure rises often returned to the resting level within 10 min and the spike-like pressure tracing curves were different from those of SPPG. We believe that the transient rise in ICP was due to the volume effect of injection of NS.

![Fig. 5. Effects of (A) triprolidine (O: control, □: 10^{-7} M, △: 10^{-6} M) and (B) SPPG (O: control, □: 1 mg/ml, △: 2 mg/ml, ▽: 4 mg/ml) on histamine evoked contraction of corpus cavernosum. (C) Triprolidine-induced relaxation of phenylephrine-precontracted cavernous strips. Each point represents the mean±s.e.m. (n = 7-9).](image-url)
Fig. 6. (A) Representative time-course changes in intracavernosal pressure (ICP, mmHg), mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) after intracavernous injection of 1 and 4 mg/kg of special processed *Panax ginseng* (SPPG) and equal volume of normal saline (NS). (B) The dose-response curves for ICP and duration of tumescence after intracavernous injection of SPPG. Each point represents the mean ± S.E.mean (N = 8).
Administration of SPPG in increasing dose (0.5, 1, 2, and 4 mg/kg) induced a dose-dependent elevation in ICP (Fig. 6B) and duration of tumescence. The peak ICP changes was increased progressively from basal to 19.2 ± 5.4, 28.7 ± 6.1, 34.6 ± 4.2 and 46.7 ± 8.2 mmHg, with a duration of 20 ± 4, 56 ± 3, 74 ± 7 and 90 ± 8 min, respectively.

DISCUSSION

Ginseng has long been reported to have aphrodisiac properties. In the present study we showed that SPPG, a special processed *Panax ginseng*, elicited a dose-dependent relaxing effect in isolated rabbit corpus cavernosum and rising of ICP in anesthetized rabbit. We also pointed out that the corporal relaxant effect of SPPG was turn out to be more potent than total ginsenosides (TG). Ginsenosides are a complex mixture of triterpene glycosides. Its effect may be caused by a single active ingredient or by the combined action of many active agents existing in this extract. Early studies on the cardiovascular effects revealed that TG, Rg1 (from 0.3 to 1.0 mg/ml) and Re (1 mg/ml) can cause endothelium-dependent relaxation in rat aorta and increased synthesis of cyclic GMP (cGMP). In contrast, Rb1 and Rc had neither action. Even though studies have been carried out previously to examine the effect of TG on rabbit corpus cavernosum tissue, however, which active ingredient(s) are involved in the corporal relaxant effect remained unclear. In the present study, HPLC analysis revealed that SPPG did not contain Rb1, Rb2, Rd, or Re, furthermore, Rg1, Rc, and Rf (26:8:1) are the predominant components. However, a reconstituted mixture with the same ratio of Rg1/Rf/Rc (26:8:1) did not mimic the corporal relaxant effect of SPPG. Our previous study demonstrated that Rg1 or Rc alone, when applied to the PE-precontracted cavernosal strips has no significant effect on the corporal cavernosum vascular tone. Even 8 mg/ml of Rg1 only evoked mild relaxation (15.8 ± 3.1%). The present results also suggested that some active component(s) other than Rg1, Re and Rf might account for the beneficial relaxant effect of SPPG. Furthermore, our results also revealed that Rg1 relaxes aortic vascular bed but not in corpus cavernosal smooth muscle with tissue selectivity.

The possible mechanism involved in SPPG was further studied by removal of endothelium. Results showed that SPPG induced corporal relaxation through an endothelium-NO independent mechanism since endothelium denudation and L-NAME treatment all failed to affect its effect.

Histamine exerts a great variety of pharmacological effects depending on the type of tissues and species involved, and is endogenously produced in many organs, including the penis. In general, histamine relaxes vascular smooth muscle cells through activation of both histamine H1 and H2 receptors while the contractile response to histamine appears to involve the activation of H1 receptors only. However, the effects of histamine on the corpus cavernosum have been poorly elucidated and inconsistently reported. Our results demonstrated that cimetidine did not induce relaxation in PE-precontracted cavernosal strips (data not shown) and did not affect contraction evoked by histamine suggesting the unlikely involvement of H2 receptors in the rabbit corpus cavernosum. Indeed, previous studies have shown variable responses to histamine in human cavernosal tissue: contraction, relaxation, or contraction followed by...
relaxation. Our results demonstrate that histamine has no significant relaxant effect on PE-precontracted tissues (data not shown), however, induced significant contractile response in the isolated rabbit corpus cavernosum through activation of H1 receptor. The suppression effect of triprolidine on histamine-induced contractile response suggested that blocking H1 receptor might have a role in regulating corporal relaxation.

Results obtained from this study can be concluded that SPPG possesses a more potent relaxant effect than TG on rabbit corpus cavernosum and was attributable to NO-independent signal pathway. The major mechanism involved in SPPG-induced relaxation is probably linked to H1 receptor antagonism property in the cavernosal vasculature. Although the active components in SPPG are not clear, its physiological effects on rabbit after intracorporeal injection implicate a potential for the treatment of erectile dysfunction. Extensive in vivo study and further identification and separation are progressing in our institution.

Widespread clinical use of histamine H1 antagonists for peptic ulcer diseases has been associated with reports of impotence. By contrast, it has been reported that histamine H1 receptor antagonists increased non-adrenergic non-cholinergic mediated corporal relaxation and possessed potential as an intracavernosal pharmacotherapeutic agent for the treatment of erectile dysfunction. Our data demonstrating that SPPG has the beneficial effect in raising intracorporeal pressure and relaxing isolated rabbit corpus cavernosum more potent than total ginsenosides possibly associated with a H1 receptor antagonizing effect. Furthermore, the results indicate that there are some other potentially active constituents in addition to major ginsenosides (such as Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1) in SPPG that could influence its final action. Further studies are required in this area to purify and select the proper components for achieving better relaxation of the corporal smooth muscle.

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