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Research article

In vitro and *in vivo* evaluation of tissue-cultured mountain ginseng on penile erection

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ABSTRACT

Background: Progressed tissue culture techniques have allowed us to easily obtain mass products of tissue-cultured mountain ginseng over 100 yr old (TCMG-100). We investigated the effects of TCMG-100 extract on erectile function using *in vitro* and *in vivo* studies.

Methods: To examine the relaxation effects and mechanisms of action of TCMG-100 on rabbit cavernosal strips evaluated in an organ bath. To investigate the long-term treatment effect of TCMG-100, 8-wk administration was performed. After administration of TCMG-100, intracavernosal pressure, cyclic guanosine monophosphate and nitric oxide (NO) levels of cavernosal tissue, serum testosterone level, histological observation of collagen fiber, endothelium, smooth muscle cell, and transforming growth factor-β1 were investigated.

Results: TCMG-100 extract displayed dose-dependent relaxation effects on precontracted rabbit corporal smooth muscle. The TCMG-100-induced relaxation was significantly reduced by removing the endothelium, and treatment with an NO synthase inhibitor or NO scavenger. Eight weeks of TCMG-100 administration increased intracavernosal pressure in a rat model. The levels of cyclic guanosine monophosphate and NO in the corpus callosum and serum testosterone level were also increased by TCMG-100 treatment. Furthermore, histological evaluation of collagen, smooth muscle, and endothelium showed increases in endothelium and smooth muscle, and a decrease in transforming growth factor-β1 expression.

Conclusion: These relaxation effects on corporal smooth muscle and increased erectile function suggest that TCMG-100 might be used as an alternative herbal medicine to improve erectile function.

1. Introduction

Erectile dysfunction (ED) has become an important health issue in terms of quality of life and the transition to an aging society. It has been reported that about 50% of men older than 40 yrs experience ED [1]. With increasing lifespan, the chance of having erectile problems increases. ED can have both physical and psychological causes including hypertension, high cholesterol, diabetes, hormonal problems, anxiety, and depression [2–4]. These causes interfere with the complicated erection process that involves contributions from nerves, muscles, blood vessels, and spongy tissue in the penis.

The pathogenesis of ED is complex. Various pathophysiological mechanisms of ED have been studied, such as cavernous nerve dysfunction, reduced production of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP), and smooth muscle or collagen degradation [5]. Among them, the reduction in levels of NO and cGMP are known to play an important role in penile ED [6].

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Therefore, pharmacological treatment of ED includes phosphodiesterase-5 (PDE5) inhibitors, which act as intracellular NO signal amplifiers by slowing the degradation of cGMP by PDE5, resulting in subsequent penile smooth muscle relaxation. However, recent reviews and studies reported the effectiveness of 5-PDE inhibitors in ED irrespective of etiology [7–10]. More attention has recently been directed toward natural alternatives to synthetic pharmaceuticals [11,12], because it seems that many people prefer to use phytotherapies rather than pharmaceutical drugs for their health.

Mountain ginseng, one of the most well-known traditional herbs, has been widely used for the healing of various disorders [13]. Since the first report of the clinical efficacy of Korean Red Ginseng for ED [14], various types of studies have demonstrated that whole-ginseng extracts or purified ginsenosides from ginseng could improve ED [15,16]. In particular, ginsenosides protect the vascular endothelium against free radical-induced injury and have a relaxing effect on vascular smooth muscle associated with NO release from the vascular endothelium [17,18]. Furthermore, ginseng has an effect on corpus cavernosal smooth muscle with no side effects [14].

Traditionally, the cultivative or economic efficacy in red ginseng production has led to the popularity of ginseng products. However, rarely gathered mountain ginseng has not gained popularity, even though mountain ginseng has unique or strong effects. Tissue culture techniques for mountain ginseng have made bioreactor technology a useful tool for large-scale production of root biomass [19]. Thus, tissue-cultured mountain ginseng over 100 yrs old (TCMG-100) is obtained from wild mountain ginseng species. Furthermore, TCMG-100 contains a specific type of ginsenoside, Re [20], different from red ginseng-contained ginsenosides, Rb1 or Rg3 [21]. The types of cells in TCMG-100 are equal to wild mountain ginseng and have genetic equivalence. However, little has been reported on the differences between wild and cultivated ginseng in the treatment of ED. Thus, we aimed to evaluate whether TCMG-100 might be an alternative to current PDE5 inhibitors for ED. We investigated the effects of TCMG-100 on isolated rabbit corpus cavernosal smooth muscle. Rats were administered TCMG-100 orally and the effect on erectile function and related parameters were subsequently assessed.

2. Materials and methods

2.1. Animals

For *in vitro* experiments, 30 male New Zealand white rabbits (2.5 ± 0.5 kg) were anesthetized with pentobarbital sodium (50 mg/kg). The rabbits' penises were surgically removed with the tunica albuginea intact. The corpus cavernosum (CC) tissue was dissected free from the tunica albuginea. The strips of CC tissues were studied in separate organ chambers. We used 50 7-wk-old Sprague-Dawley rats for *in vivo* experiments. They were allowed to adapt for 1 wk before use and were permitted access to food and water *ad libitum*.

The animals were purchased from Koa-tech (Seoul, Korea) and maintained at a temperature of 20 ± 2°C, humidity of 45 ± 10%, and a 12-h light/dark cycle. Experimental animals were handled according to principles outlined in the Guide to the Care and Use of Experimental Animals Prepared by the Chung-Ang University Committee of Animal Ethics (Seoul, Korea).

2.2. Preparation of TCMG-100 extract

TCMG-100 was kindly provided from Omnica (Tokyo, Japan) and cultured by JIN-SANSAMBIO (Seoul, Korea). Briefly, callus, induced from wild mountain ginseng tissue, was cultivated to the adventitious root. The adventitious roots were selected and cultivated in a sterile bioreactor for about 90 d. The cultivated TCMG-100 was qualified using a UV method of saponin-component ratio quantification (> 40 mg/g). The dried TCMG-100 was extracted with 70% ethanol and the concentrated under rotary evaporator. All extracts were freeze-dried cycle to yield of extract powders. Thus, extracted powder was used to carry out *in vivo* or *in vitro* experiments.

2.3. High-performance liquid chromatography analysis

The high-performance liquid chromatography conditions were based on those described by Kanazawa et al [22], which provided satisfactory resolution of major ginsenosides including Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1. The ginsenoside separation was conducted on a Eurospher C18 5 μm 200 × 3 analytical fractions column (Altmann Analytik, München, Germany) using the following gradient system: 0–25 min, 17% acetonitrile, and 83% distilled water (DW); 25–50 min, 25% acetonitrile, and 75% DW; 50–105 min, 40% acetonitrile, and 60% DW; 105–125 min, 100% acetonitrile; 125–135 min, 17% acetonitrile, and 83% DW. The flow rate was 0.8 mL/min, and the ginsenosides were monitored at 203 nm. The sample injection quantity was 10 μL, and the temperature of the column was sustained at 30°C. The ginsenoside peaks were monitored, with the peak areas corresponding to samples matching authentic ginsenoside standards purchased from ChromaDex (Santa Anna, CA, USA).

2.4. In vitro experiments

Organ bath tissue experiments were performed using the methods described by Choi et al [23]. Briefly, the strips of rabbit CC measuring approximately 2 mm × 2 mm × 6 mm were mounted longitudinally in a 20 mL organ bath chamber containing Krebs buffer solution with 95% oxygen and 5% CO_2 gas. The CC was stretched for 1 h and the optimal resting isometric tension for contraction was determined. The tissue was contracted with phenylephrine (PHE) 5×10^{-6}M after every stretch (0.5 g, tension/stretch). Each strip was used for up to four separate rounds of testing, washed three times with Krebs solution, and allowed to equilibrate for 30 min between rounds.

Relaxation was studied in muscle strips precontracted with PHE. After muscle strips precontracted with PHE were stabilized, they were treated with solutions of TCMG-100 in increasing concentrations from 5 mg/mL. The mechanism of muscle relaxation

Table 1
Contents of ginsenosides in the tissue-cultured mountain ginseng over 100 yr old (w/w%)

Ginsenosides	TCMG-100
Rb1	0.703 ± 0.084
Rb2	0.683 ± 0.036
Rc	0.802 ± 0.067
Rd	0.345 ± 0307
Re	2.066 ± 0.036
Rf	0.211 ± 0.003
Rg1	0.059 ± 0.030
Rg2	0.178 ± 0.030
Rg6	0.006 ± 0.005
Rh1	0.050 ± 0.008
F1	0.046 ± 0.020
Total ginsenosides	5.670
Diol/triol	0.969

Data are presented as mean ± standard deviation.
TCMG-100, tissue-cultured mountain ginseng over 100 yr old.

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