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EXPERIMENTAL STUDY

Estrogenic effect of the extract of Renshen (*Radix Ginseng*) on reproductive tissues in immature mice

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Abstract

OBJECTIVE: To evaluate the estrogenic efficacy of Renshen (*Radix Ginseng*) (GS) on reproductive target tissues in immature mice.

METHODS: One hundred and ten female immature Kunming (KM) mice, 21-day-old, were randomly assigned to eleven groups, 10 for each; one served as control group treated with 0.154 mg/kg estradiol valerate (EV, n = 10), the rest were treated respectively with GS intragastrically at a daily dose of 0.5, 1.0, 1.5, 3.0, 6.0, 12.0, 18.0, 24.0 and 30.0 g/kg (n = 10 in per group) for 7 days. The estrous cycle, uterine weight, hormone levels in circulation and histomorphology changes of uterus and vagina were scrupulously examined. The estrogen receptor (ER) α and ERβ expressions in the uterus and vagina were detected by immunohistochemistry and western blotting.

RESULTS: Treatment with GS at the dose of 12.0, 18.0 and 24.0 g/kg resulted significant estrogenic activity in the mice, as indicated by advanced and prolonged estrous stage and increased uterine weight (all $P < 0.05$). GS treatment substantially promoted development of reproductive tissue by thickening the uterine endometrium and increasing vaginal epithelial layers. In addition, treatment with GS induced significant up-regulation of ERα and ERβ expressions in reproductive tissues, and ERα up-regulation was stronger than that of ERβ. GS could raise levels of circulating estrogen, simultaneously decrease levels of luteinizing hormone and follicle-stimulating hormone (all $P < 0.001$) compared with the control group.

CONCLUSION: Our findings suggest that GS had estrogenic effect on reproductive tissues in immature mice by stimulating biosynthesis of estrogen in circulation and up-regulating ERs.

Key words: Panax; Uterus; Vagina; Receptors, estrogen; Follicle stimulating hormone; Luteinizing hormone

INTRODUCTION

Renshen (*Radix Ginseng*) (GS) is an herbal medicine

that has been used for over 2000 years in oriental countries. It has been reported that GS has a wide range of pharmacological activities in cardiovascular, endocrine, immune, and central nervous systems.[1] Studies also showed that GS could relieve menopausal symptoms, bleeding disorders, sleeping disorders, depression and anxiety, which indicateds that some components of GS act as phytoestrogens and/or involve activation of the estrogen receptor (ER).[2-6] *In vitro* studies revealed GS extracts were able to stimulate the growth of ER-positive cells.[7] Ginsenoside-Rg_1, -Rb_1 and -Rh_1 were the major estrogen active compounds of ginsenoside.[8-11] Rg_1 was shown to be a potent phytoestrogen that preferentially activated ERα *via* phosphorylation of the activation function-1 domain in the absence of receptor binding.[11] Nowadays, most studies focus on mechanism of GS in vitro. However, the estrogenic actions of GS on reproductive tissues and the underlying mechanism are not fully addressed,besides Wang *et al* [12] reported that administration of Rb_1 caused an increase uterine weight of the normal female mouse. In the present study, we aimed to investigate the estrogenic effects of GS on reproductive target tissues of immature mice by monitoring estrous cycle, uterine weight gain, hormone levels, observing the histological structure changes and ERαand ERβ expressions, as part of an ongoing effort to provide scientific data and identify potent estrogen-like activity of GS.

MATERIALS AND METHODS

Animals and experimental design

Totally 110 of 21-day-old female immature mice (12 ± 2) g were purchased from Experimental Animal Center of Academy of Military Medical Sciences (Certificate of quality No. SCXK [jun] 2007-004). The mice were randomly divided into eleven groups (10 for each) by random number table method; one was the control group treated with 0.154 mg/kg estradiol valerate (EV); the rest were treated respectively with GS intragastrically at a daily dose of 0.5, 1.0, 1.5, 3.0, 6.0, 12.0, 18.0, 24.0 or 30.0 g/kg for 7 days. Dose calculations followed guidelines correlating dose equivalents between humans and laboratory animals, on the basis of ratios of body surface area.[13] Untreated control mice received distilled water only. All animals were maintained on a 12-h light/dark cycle under constant temperature (24 ± 2) ℃and humidity (55% ± 5%), and were allowed free access to food and water. All procedures for consideration of animal welfare were reviewed and approved by the ethical committee of China Academy of Traditional Chinese Medicine.

Chemicals and reagents

Estradiol valerate (EV) was purchased from Bayer Schering Pharma (Berlin, Germany). ERα antibody (MC-20) was purchased from Abcam Biotechnology (Cambridge, UK). ERβ antibody (ab3577) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (14C10) was purchased from Cell Signaling (Boston, MA, USA). All other chemicals were of analytical grade.

The preparation of GS extract

GS was purchased from Changchun Medicinal Herbs Co., Ltd., (Jilin, China) and identified and authenticated by an expert at Changchun Institute of Applied Chemistry Chinese Academy of Sciences. GS was pulverized to a fine powder and boiled twice with distilled water for 1 hour under reflux. The aqueous extracts were collected and filtered. The filtrates were then concentrated under reduced pressure at 50℃ and to a concentration of 0.6 g/mL. As previous descripted[14] that the representative chemical compositions of Rb_1 (0.228%), Rf (0.252%), Rc (0.252%), Rg_2 (0.636%), Rd (0.06%), Rg_1 and Re (0.456%) in the GS extract were determined by high performance liquid chromatography (HPLC) analysis. The total ginsenosides, calculated as the sum of the above individual ginsenosides, represented 1.884% in the GS extract.

Monitoring estrus cycle

All mice were monitored by daily vaginal epithelium cells smear testing during the 7 days administration period. The vaginal lavage was fixed with 95% ethanol for 10 min and stained with methylene blue for 10 min.[15] Vaginal epidermal cells were observed by Olympus OX31 microscopy (Olympus, Tokyo, Japan), and keratinized vaginal cells were taken as being indicative of estrus.

Analysis of tissue and serum

Blood was collected from the eye venous plexus and animals were sacrificed by decapitation after 7 days of treatment. The serum were analyzed for estradiol (E_2), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by enzymelinked immunosorbent assay (ELISA). The uterus and vagina were removed and weighed. The left horns of the uterus and the upper portion of vagina were stored at − 80℃. The right horns of the uterus and the under portion of vagina were fixed with 4% polyoxymethylene for 24 h. All samples were embedded in paraffin and prepared for cross sections; sections 4 mm thick were cut, mounted, and stained with Hematoxylin & Eosin (HE) for microscopy (Olympus, Tokyo, Japan).[16]

Immunohistochemistry

Tissue sections 4 μm thick of uterus and vagina were mounted on polylysine-coated slides, deparaffinated, rehydrated, and then heated with 10mM citrate buffer, pH 6.0. After two washes with phosphatebuffered saline (PBS), slides were incubated with 3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity. After washing with PBS, tissues were incubated with blocking serum (Boster