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Fructus panax ginseng extract promotes hair regeneration in C57BL/6 mice

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ABSTRACT

Ethnopharmacological relevance: Radix panax ginseng (*Panax ginseng* C.A. Meyer, Araliaceae, RPG) has been documented to possess hair growth activity and widely used to treat alopecia, while no report has been issued to date on the effect of *Fructus panax ginseng* (FPG) on hair regeneration.
Materials and methods: To investigate the effects of FPG extract on the proliferation of human hair dermal papilla cells (DPCs) and on the promotion of hair regeneration in C57BL6 mice, cell proliferation was evaluated in cultured DPC by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and measured the expressions of Bcl-2 and Bax by immunoblot assay. We also compared the effects of topical FPG extract (1 and 10 mg/ml, 100 μl/d) with the effects of minoxidil as a positive control (5%, 100 μl/d) or vehicle control (30% ethanol) on the depilation-induced hair cycling in 7 week-old-C57BL/6 mice.
Results: FPG extract significantly increased the proliferation of DPCs in dose and time dependent manners ($P < 0.05$, $P < 0.01$ and $P < 0.001$). FPG extract also enhanced Bcl-2 expression and decreased Bax expression compared with control ($P < 0.01$). Moreover, significant elongations of anagen phase during hair cycle after application of FPG were evaluated by photographical and histological observations.
Conclusions: FPG extract improves the cell proliferation of human DPCs through anti apoptotic activation. Topical administration of FPG extract might have hair regeneration activity for the treatment of hair loss.

1. Introduction

Hair loss or alopecia is a chronic inflammatory dermatological disease. It affects the hair follicles (HFs) and is characterized by a decrease in anagen HF, and miniaturization of the HF (Ellis et al., 2002), in which people lose some or all of the hair on their head and sometimes on their body as well (Hunt and McHale, 2005). An increasing number of people have been suffering from hair loss or alopecia (Paik et al., 2001; Krescher et al., 2007). Although two FDA-approved hair loss drugs, finasteride and minoxidil, are available, their uses are limited and transient because of unpredictable efficacy and side effects. Therefore, more and better treatment options are urgently needed (Paus, 2006).

Hair growth is controlled by cyclic bouts of degeneration (catagen), rest (telogen) and regeneration (anagen) in HFs over the entire lifetime of a mammal (Greco et al., 2009). The dermal compartment of the HF consists of the dermal papilla and dermal sheath (Yang and Cotsarelis, 2010). Human hair dermal papilla cells (DPCs) have an essential function in the regulation of hair growth not only in the normal hair cycle but also in the pathogenesis of certain conditions, for example, in androgenetic alopecia (Yang and Cotsarelis, 2010). Studies have shown that size of DPC is well correlated with hair growth cycle, and the number of DPC is increased in anagen phase (Elliott et al., 1999).

Radix panax ginseng (*Panax ginseng* C.A. Meyer, Araliaceae, RPG) has been documented to possess hair growth activity in traditional medicine (Marderosian and Liberti, 1998; Choi et al., 2003). It has been reported that *Panax ginseng* promoted hair growth on cultured mouse vibrossal HF (Matsuda et al., 2003) or prevented apoptosis in HF and accelerated recovery of hair medullary cells in irradiated mice (Kim et al., 1998). RPG has already been added to hair treatment products as a safe ingredient (Kim et al., 1989, 2009). Recent clinical trial found that oral consumption of Korean red ginseng extract (3000 mg/d) for 24 wks effectively increased hair density and thickness in alopecia patients (Kim et al., 2009). However, no report has been issued on the effect of FPG on hair growth.

In this study, the effects of FPG on proliferation and apoptosis of human hair DPCs were investigated. Furthermore, the

Abbreviations: DPC, Dermal papilla cell; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; FPG, *Fructus panax ginseng*; HFs, Hair follicles; RPG, *Radix panax ginseng*; MSCM, Mesenchymal stem cell medium; FBS, Fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.

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promotion effect of the topical FPG extract on the hair regeneration was demonstrated in C57BL/6 mice.

2. Materials and methods

2.1. Materials

FPG was obtained from Keumsan, Korea. About 100 g of FPG was extracted with 1000 ml of 95% ethanol at 65 °C for 120 min, consecutively. The extract was then concentrated using vacuum evaporator. The weight of the resulting residue was determined as 18.6 g, and it was then dissolved in 30% ethanol for subsequent treatment for *in vivo* study. FPG extracts for *in vitro* study were then filtered to sterilize using 0.45 μm-filter units (Millipore, MA, USA).

Components were identified by Professor Sung Kwon Ko, Department of Oriental Medical Food and Nutrition, Semyung University, Jechoen, Korea, as previously described (Ko et al., 2008). Standards of various ginsenosides (99% purity) were obtained from Chromadex (St. Santa Ana, CA, USA). Total ginsenoside content of FPG was 9.09%. Ginsenoside Re (5.99%) was revealed as the major component of FPG extract. Minoxidil was obtained from Hyundai Pharm. Co. Ltd. (Cheonan, Korea). MTT was obtained from Sigma (St. Louis, MO); it was dissolved in 0.12 M HCl at 1 mM and stored at −20 °C. Antibodies recognizing Bcl-2 and Bax were obtained from Dako (Glostrup, Denmark); β-actin antibody from Santa Cruz Biotech Inc. (Santa Cruz, CA).

2.2. Cells and growth condition

DPCs were purchased from ScienCell (Calsbad, CA) as primary cells and grown in mesenchymal stem cell medium (MSCM) supplemented with 5% fetal bovine serum (FBS), 1% penicillin, and 1% mesangial cell growth supplement in a humidified environment at 37 °C in 5% CO_2. Media, FBS and other chemicals were obtained from ScienCell (Calsbad, CA).

2.3. Cell viability assay

Subcultured human DPCs were used within third passages for the MTT assay and cell growth was evaluated according to the method of Randall et al. (1991). In brief, DPCs were seeded into 96-well plates at 1×10^4 cells/ml in triplicate and incubated for 24 h. And the media were changed to Dulbecco's modified Eagle's medium (DMEM) (Sigma, CA), and then treated and incubated with 10 μl of FPG extract with various concentrations (0.8, 4, 20, 100, 500 μg/ml) for 24 h, 48 h, and 72 h, respectively. Absorbance was measured at 570 nm using a plate reader (Bio-Tek, Vermont). Results were expressed as percentages of untreated controls in three cultures. Cell numbers and their viability were determined by trypan blue exclusion assay.

2.4. Immunoblot analysis

Total proteins were separated by 12% SDS-PAGE gels, and transferred to PVDF membrane using a semidry transfer system (Hoeffer Phamacia Biotech, San Francisco, CA). Membranes were blocked in 5% non-fat dry milk and probed with specific antibodies at appropriate dilutions corresponding to Bcl-2 (1:1000), Bax (1:1000) and β-actin, respectively. The membranes were washed three times and incubated with a horseradish peroxidase-conjugated secondary antibody, developed using a commercial enhanced chemiluminescence system (ECL, Amersham, UK), and exposed to films, and results were analyzed using Bio-Rad GS-700 imaging densitometer (Hercules, CA).

2.5. Hair growth activity in vivo

All experimental protocols were approved by the animal care and use review committee of Semyung University (Certified protocol number: SMECAE 091101). Male six-week-old C57BL/6 mice ($n = 40$) purchased from SLC (Shizuoka, Japan) were housed individually in stainless steel cages in a room with controlled temperature (22 °C ± 2 °C), a relative humidity of 55 ± 5%, and a 12 h cycle of light and dark. Mice were maintained on a commercial pellet diet (Semtaco, Seoul, Korea) and fresh tap water. After acclimatization for 7 days, all mice were anesthetized with an intraperitoneal injection of rumpun (Bayer, Leverkusen, Germany) and zoletil (Virbac, Carros, France) mixture. The dorsal areas (approximately 2 cm in width and 4 cm in length) of the mice were clearly removed using wax-rosin mixture according to the method of Chase (1954). Mice were randomly allocated into three experimental groups of ten animals each and provided with one of the following treatments: 30% ethanol as vehicle for negative control group, 5% minoxidil as positive control group, and 1 mg/ml FPG extract as experimental group. All mice were topically applied to the test area with 100 μl every day for 21 days. The back skin of mice were observed and photographed and then isolated to examine histological features at day 1, 4, 7, 14, and 21 after depilation. Individual skin samples were fixed in 4% paraformaldehyde (Sigma–Aldrich, MO, USA), and the tissues were then dehydrated and embedded in paraffin using an automatic tissue processor (Leica ASP 300; Leica Microsystems, Wetzler, Germany), sectioned to 4 μm thickness with a microtome, stained with hematoxylin and eosin, and then histological morphology was examined using light microscopy (Olympus CX 31; Olympus Tokyo, Japan).

2.6. Statistical analysis

Results are presented as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test ($P < 0.05$, 0.01, or 0.001).

3. Results

3.1. Effect of FPG extract on human hair dermal cell growth

The potential of FPG extract on the growth of DPC was evaluated by the cell viability and cell growth assay. During the treatment of FPG, cell shape was not changed (data not shown) and cytotoxicity of FPG was not detected. FPG treatment resulted in significant DPCs proliferation by 128–135% compared with the vehicle-treated controls during the 24 h incubation as shown in Fig. 1. FPG extract

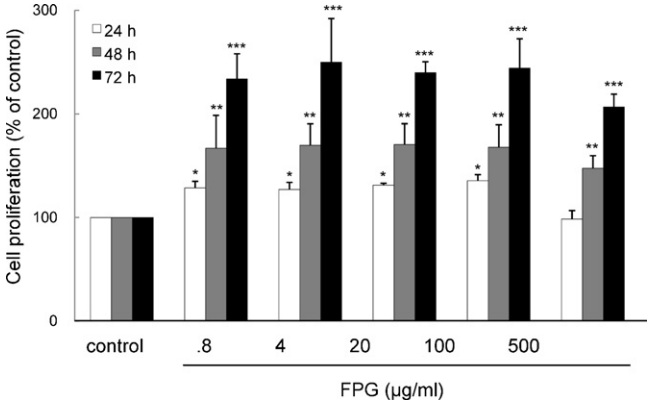

Fig. 1. FPG extract enhanced the proliferation of cultured human dermal papilla cells as determined by MTT assay. Values are means ± SD; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the vehicle-treated control.