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Effect of Red Ginseng Total Saponins on the Hair Follicles

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Background: Red ginseng is one of the most popular medicinal plants in oriental medicine, and it has been well known that ginseng has a variety of activities against many diseases. Purpose: In the present study, effect of red ginseng total saponins (RGTS) on the hair follicles was examined. **Methods:** Human hair follicles and mouse vibrissa follicles were cultured organo-typically and the effect on the growth of hair follicles was measured using [^{35}S]cysteine uptake method. The DNA synthesis in human hair dermal papilla cells was also investigated using [^{3}H]thymidine incorporation assay. The effect on the expression level of insulin-like growth factor-1 (IGF-1), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in dermal papilla cells was measured by semi-quantitative RT-PCR. **Results:** RGTS increased the [^{35}S]cysteine uptake in both human scalp hair and mouse vibrissa follicles cultured *in vitro*. In addition, RGTS increased DNA synthesis in human hair dermal papilla cells cultured *in vitro*. However, RGTS had no effect on the gene expression of growth factors such as IGF-1, KGF, HGF and VEGF in dermal papilla cells. **Conclusion:** These results suggest that RGTS may have impact on the hair growth.

Key words : Red ginseng total saponins, Hair growth, Hair follicle organ culture, Dermal papilla cells

INTRODUCTION

Hair grows in cyclic manner that is characterized by a finite period of hair fiber production (anagen), a brief regression phase (catagen), and a resting period (telogen)[1-3]. Recently, the number of men and women who suffered from hair loss and/or hair thinning is increasing in accordance with the change of life style and nutritional balance. Thus, it is of great importance to develop new therapeutic materials to stop hair loss and to enhance hair growth. Many researchers have concentrated their efforts on finding the therapeutics for hair loss treatment. Among those, complementary and alternative (CAM) medicine is one interesting field of study. Despite the limited scientific evidences and lack of mechanistic understanding, CAM has been increasingly attractive around the world because of traditional belief of both safety and efficacy[4]. For instance, bisbenzylisoquinoline alkaloids from Stephania cepharantha, Polyporus umbellatus Fries extract, and Sophora flavescens extract were reported as possible agents to induce hair growth[5-7].

Red ginseng is a widely used medicinal plant in oriental medicine, and it has been well known that ginseng has a variety of activities against many diseases. These include antitumoral, antidiabetic, immuno-modulatory and epidermal proliferative activities[8-11]. Furthermore, it has been also reported that ginseng prevents apoptosis in hair follicles and accelerates recovery of hair medullary cells in irradiated mice[12]. The potential effect of red ginseng on the hair growth, however, has not been well elucidated yet. In the

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present study, we have investigated the effect of red ginseng total saponins (RGTS) on the hair follicles using cell and organ culture systems.

MATERIALS AND METHODS

Materials

RGTS was obtained from Korea Ginseng & Tobacco Research Institute. Dulbecco's modified Eagle's medium (DMEM), Williams E medium and fetal calf serum (FCS) came from Gibco BRL (Gaitherburg, MD, USA), [methyl-^{3}H]thymidine and [^{35}S]cysteine from Amersham Pharmacia Biotech (Buckinghamshire, UK). C57BL/6 mice were supplied from Dae-Han Biolink (Eumsung, Chungbuk, Korea). M-MLV reverse transcriptase and Taq polymerase were purchased from Promega (Madison, WI, USA).

Organ culture of hair follicle and [^{35}S]cysteine uptake

The human anagen hair follicles were isolated and cultured as previously described method[13]. Briefly, scalp skin specimens were obtained from plastic surgery of normal men aged from 25 to 30, and isolation of hair follicles was achieved by using a scalpel blade to cut through the skin at the dermo-subcutaneous fat interface. The intact hair follicle was removed under a stereomicroscope using watchmaker's forceps, by gently gripping the outer root sheath (ORS) of the follicle and pulling out of subcutaneous fat. Isolated hair follicles were maintained free floating in Williams E medium supplemented with 10 ng/mL insulin and 10 ng/mL hydrocortisone at 37℃ in an atmosphere of 5% CO_2. Mouse vibrissa organ culture was conducted according to previously reported method[14]. Vibrissa follicles were carefully dissected from upper lip pad of C57BL/6 mice and maintained in Williams E medium supplemented with 10 ng/mL insulin and 10 ng/mL hydrocortisone. The rate of protein synthesis in isolated hair follicles was determined by measuring [^{35}S]cysteine uptake. Follicles were incubated in the presence of 1 μCi of [^{35}S] cysteine and RGTS at the indicated concentrations. After washing twice with PBS, follicles were lysed with tissue solubilizer (Beckman) at 56℃ for 1 h and the radioactivity was measured by liquid scintillation counter (Beckman).

Culture of human hair dermal papilla cells and [^{3}H]thymidine incorporation

The human hair dermal papilla (DP) cells were cultured according to the method previously described[15] with slight modifications. The DP was isolated under a stereomicroscope using watchmaker's forceps and transferred into DMEM supplemented with 10% FCS. DP cells were outgrown normally for 5 - 7 days after explanting. Cultures were maintained at 37℃ in a humidified atmosphere containing 5% CO_2. The third or fourth passage DP cells were used in this study. For [^{3}H]thymidine incorporation assay, the DP cells were plated at 5×10^4 per 60-mm culture dishes and grown in DMEM supplemented with 10% FCS for 24 h. After washing twice with PBS, cells received DMEM without FCS, 1 μCi of [^{3}H]thymidine and RGTS at the indicated concentrations. Following incubation for 24 h, cells were washed twice with PBS, once with 5% cold TCA, and then lysed with 0.1 N NaOH, 1% SDS. The radioactivity was then measured by liquid scintillation counter.

Reverse transcription-polymerase chain reaction (RT-PCR)

The DP cells were grown on 100-mm tissue culture dishes to about 80% confluency in DMEM supplemented with 10% FCS. After washing twice with PBS, DP cells were cultured with DMEM without FCS and RGTS at the indicated concentrations for 18 h. Total RNAs were extracted by acid guanidinium thiocyanate-phenol-chloroform method[16]. Two μg of total RNAs were reverse transcribed with M-MLV reverse transcriptase in the presence of random hexamer. The resultant RT mixtures were then subjected to PCR cycles as follows: 94℃ for 30s, 58℃ for 30s, 72℃ for 60s for 35 cycles (IGF-1, HGF and VEGF) and 30 cycles (KGF). Primers for amplifying the respective fragments are listed in Table 1. After agarose gel electrophoresis, PCR products were quantified using a densitometer (Imagemaster, Pharmacia Biotech).

Statistical analysis

Data for [^{35}S]cysteine uptake and [^{3}H]thymidine incorporation assay were statistically evaluated using Student's t-test. Statistical significance was set at $p<0.05$.

RESULTS

Effect of RGTS on the growth of follicle tissues cultured *in vitro*

For testing the effect of RGTS on the hair growth *in vitro*, we have established organ cultures of human scalp hair follicle and mouse vibrissa follicle (Fig. 1A, B). Over the 10 days, isolated human hair follicles increased in length by approximately 0.25 mm per day (Fig. 1C). During this time, hair bulbs maintained normal anagen morphology, and elongation occurred by an increase in length of the hair fiber. This was well reflected by [^{35}S]cysteine uptake assay (Fig. 1D), since the major structural proteins of hair fiber are cysteine-rich keratins[17]. To evaluate the hair growth stimulating effect, the follicles were cultured in the presence of [^{35}S]cysteine and RGTS at the indicated concentrations. As shown in Fig. 2A, RGTS significantly increased the uptake of radiolabeled cysteine in the human hair follicles, resulting in 117% uptake relative to control at the dose of 0.0001%. The effect of RGTS was also reflected in mouse vibrissa follicles cultured *in vitro*, which showed 115% of [^{35}S]cysteine uptake compared to control at the dose of 0.0001% (Fig. 2B). Since it has been defined that [^{35}S]cysteine uptake is well correlated to hair follicle growth cultured *in vitro*[14], these results imply that RGTS may produce stimulating effect on the hair growth.

Effect of RGTS on the cultured dermal papilla cells

The mesenchyme-derived DP cells play a pivotal role in the hair growth regulation[18,19]. Evidence have shown that the size of DP is well correlated with hair growth and the cell number of DP is increased in growing phase of hair

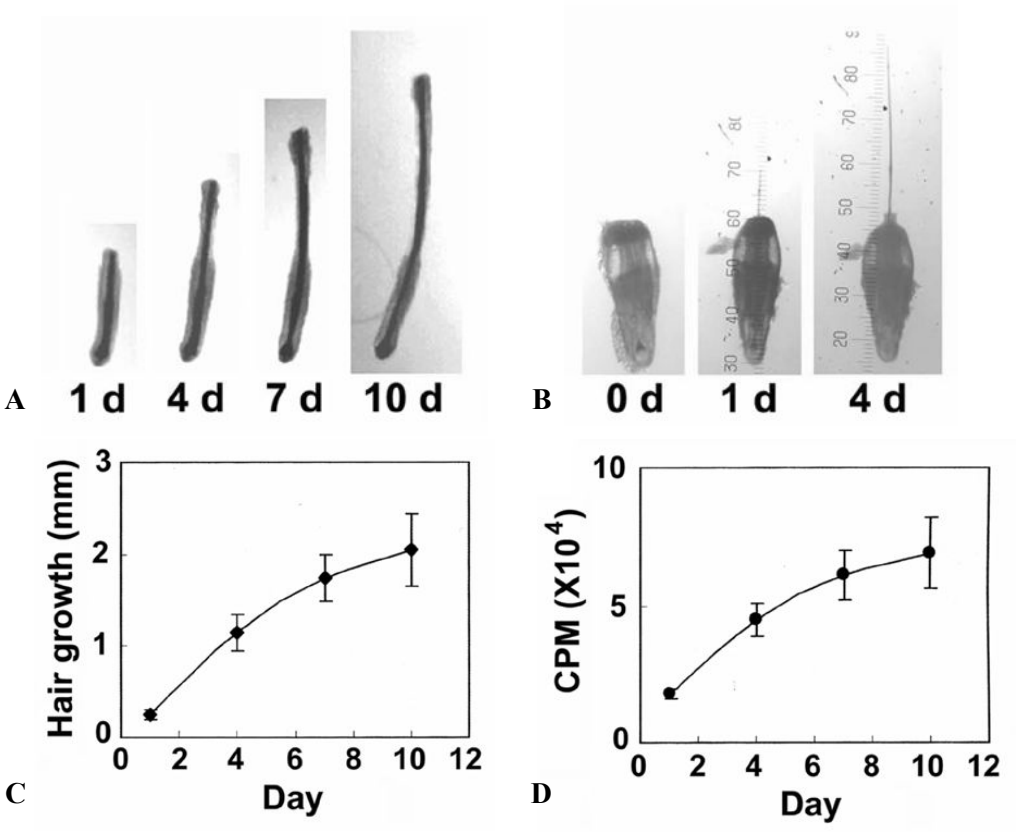

Fig. 1. Establishment of human hair follicle organ cultures *in vitro*. Human hair follicles (A) and mouse vibrissa follicles (B) were isolated and cultured in Williams E medium supplemented with insulin and hydrocortisone. (C) Growth rate of human hair follicle cultured *in vitro*. The length of hair follicle in culture was measured using a microscope with the reticle. (D) [^{35}S]cysteine uptake analysis. Hair follicles were incubated in the presence of 1 μCi of [^{35}S]cysteine, lysed and radioactivity was measured by liquid scintillation counter. All experiments were done with 30 - 40 hair follicles, and the results are represented as the mean values ± SE of triplicate measurements.

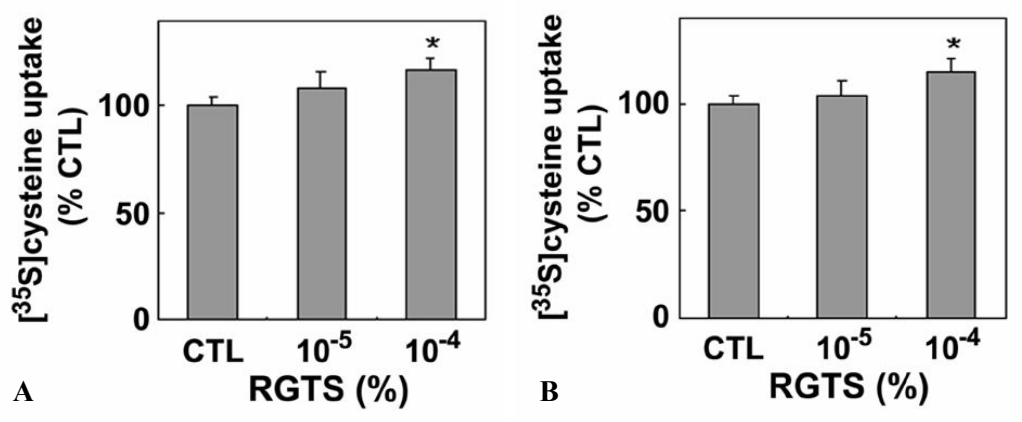

Fig. 2. Effect of red ginseng total saponins (RGTS) on the growth of follicle tissues cultured *in vitro*. (A) Human hair follicles were isolated from the scalp specimen and cultured in the presence of [^{35}S]cysteine for 7 days. (B) Mouse vibrissa follicles were dissected under a stereomicroscope and cultured in the presence of [^{35}S]cysteine for 4 days. RGTS was added at indicated concentrations. Follicles were then lysed with tissue solubilizer and the radioactivity was measured by liquid scintillation counter. The amount of [^{35}S]cysteine uptake was expressed as percentage of control. All experiments were done with 30 - 40 hair follicles per each group, and the results are represented as mean values ± SE of quadruplicate measurements (*p<0.05 vs. control).

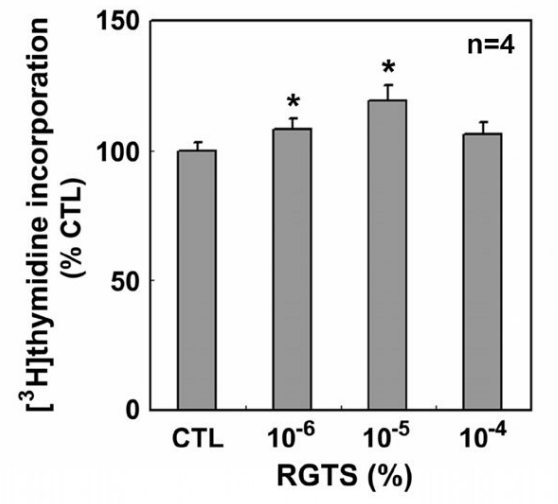

Fig. 3. Effect of red ginseng total saponins (RGTS) on the growth of human hair dermal papilla (DP) cells cultured *in vitro*. The DP cells were cultured in the presence of [^{3}H]thymidine for 24 h. The DP cells were lysed with 0.1 N NaOH, 1% SDS and the radioactivity was measured by liquid scintillation counter. The amount of [^{3}H]thymidine incorporation was expressed as percentage of control. The mean values ± SE are averages of quadruplicate measurements (*p<0.05 vs. control).

cycle[20]. These previous findings led us to examine whether RGTS has stimulatory effect on the growth of human hair DP cells cultured *in vitro*. As shown in Fig. 3, RGTS increased [^{3}H]thymidine incorporation by 119% at the dose of 0.00001% and 108% at the dose of 0.000001% as compared with control group. This result suggests that the hair follicle stimulating effect of RGTS may be mediated, in part, through the mitogenic effect on the DP cells.

Effect of RGTS on the expression of growth factors in dermal papilla cells

It has been well recognized that the DP secrets diffusible factors and affects the follicular epithelium such as matrix and outer root sheath1[19,21]. To investigate the effect of RGTS on the expression of several growth factors that were implicated in the regulation of hair growth, we performed semi-quantitative RT-PCR analysis. RGTS had no effect, however, on the mRNA levels of insulin-like growth factor-1 (IGF-1), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in the dermal papilla cells (Fig. 4).

DISCUSSION

The difficulties in developing the effective therapeutics for hair growth lie in the fact that a single proper evaluation method has not yet been established. The most relevant approach for assessing hair growth is obviously human clinical trials. But it is actually impossible to screen

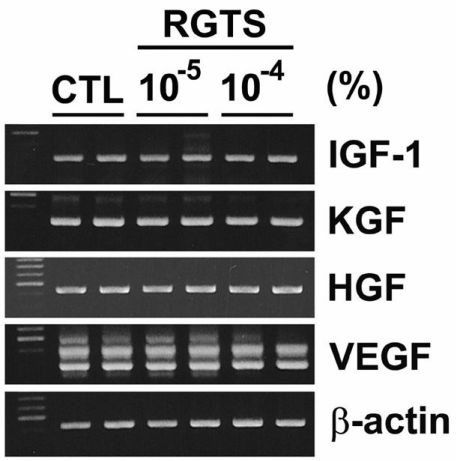

Fig. 4. Effect of red ginseng total saponins (RGTS) on the mRNA level of growth factors in cultured human hair dermal papilla cells by semi-quantitative RT-PCR analysis. The DP cells were cultured with RGTS as indicated concentrations for 18 h, then total RNAs were isolated and RT-PCR was performed.

the effective materials using human clinical test; it takes a long time and is very expensive. Thus, efforts have been made to exploit the appropriate model systems, which reflect the regulation of hair growth. The great success of hair follicle organ culture has provided valuable tools for assessing hair growth. In 1990, Philpott et al. demonstrated that human hair follicle could be cultured organo-typically *in vitro*[13]. In this approach, human scalp skin follicles were truncated below the dermis, dissected free of dermal and subcutis tissues and placed in serum free culture condition. Using similar experimental technique, the culture of hair follicle from multiple other species, such as rat, sheep and horse, has also been successfully established[22-24]. And many investigators have adopted organotypic hair follicle culture models to evaluate the effects of several compounds[14,25,26].

In the present study, we have demonstrated that RGTS has the hair follicle stimulating effect using above-mentioned organ culture systems. When the human hair follicle tissues and mouse vibrissa follicles were treated with RGTS, the cysteine uptake is significantly increased as compared with control group. As the main components of hair shaft are the cysteine-rich keratin filaments, these results suggest that RGTS has a positive effect on hair fiber formation. In addition, RGTS showed stimulatory effect on the proliferation of human hair dermal papilla (DP) cells cultured *in vitro*. The DP is a condensate of mesenchyme-derived cells and plays an important regulatory role in determining the type of hair produced1. The morphology of the DP can be altered through the hair growth cycle, being maximal in volume in growing phase (anagen) and least at resting phase (telogen). This is, in fact, a result of changes in cell number and amount of extracellular matrix (ECM) within the DP[19,20]. Thus, the proliferative potential of DP is thought to be one of important parameters that regulate the hair growth. In this regard, it is of note that RGTS has stimulatory effect on the proliferation of DP cells.

The important roles of growth factors in the hair growth are well recognized. Several lines of evidence have shown that the DP plays a pivotal role through the secretion of diffusible factors. For instance, IGF-1 was reported to be expressed in the DP and to stimulate the growth of follicular epithelial cells[27,28]. Moreover, in IGF-1 transgenic animals, hair elongation was significantly increased as compared with their littermates[29]. KGF, an important paracrine mediator of proliferation and differentiation in a wide variety of epithelial cells, was implicated in the hair growth regulation since KGF directly affects the development of hair follicles in transgenic mice model[30]. It has been also reported that HGF and VEGF have stimulatory effect on hair follicle growth[31-33]. Accordingly, it is regarded that the regulation of growth factors in the DP cells is fundamental to the growth control of hair follicle. In this study, RGTS had no effects, however, on the mRNA level of growth factors, suggesting that the hair follicle stimulating effect of RGTS may be, in part, through the mitogenic effect rather than the regulatory effect on the gene expression of growth factors in the DP cells.

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