

# Promotion of Hair Growth by Ginseng Radix on Cultured Mouse Vibrissal Hair Follicles

Hideaki Matsuda<sup>1\*</sup>, Miho Yamazaki<sup>1</sup>, Yusuke Asanuma<sup>2</sup> and Michinori Kubo<sup>1</sup>

<sup>1</sup>Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1, Kowakae, Higashiosaka, Osaka, 577-8502, Japan

<sup>2</sup>Daiichi Pharmaceutical Co., Ltd., 3-14-10, Nihonbashi, Chuo-ku, Tokyo, 103-8234, Japan

**A 70% methanol extract from red ginseng (steamed and dried roots of *Panax ginseng* C. A. Meyer, a kind of Ginseng Radix) had superior activity to that of white ginseng (peeled and dried root of *P. ginseng*, another kind of Ginseng Radix) in a hair growth promoting assay using mouse vibrissal follicles in organ culture. Of the major constituents of *P. ginseng*, ginsenoside-Rb<sub>1</sub> (G-Rb<sub>1</sub>) exhibited activity, but ginsenoside-Rg<sub>1</sub> (G-Rg<sub>1</sub>) and -Ro (G-Ro) were ineffective. Additionally, 20(S)-ginsenoside-Rg<sub>3</sub> (20(S)-G-Rg<sub>3</sub>) formed by the processing of red ginseng from the crude root of *P. ginseng* also showed hair growth promoting activity. These results indicate that Ginseng Radix possesses hair growth promoting activity, and its bioactive components are partially attributable to the ginseng saponin components mentioned above. Copyright © 2003 John Wiley & Sons, Ltd.**

*Keywords:* *Panax ginseng*; Ginseng Radix; ginsenoside; hair growth activity; organ culture.

## INTRODUCTION

Ginseng Radix is an important crude drug that has been used from ancient times to improve constitutional tendencies to poor body condition, to promote appetite, to increase vitality and to reduce over-sensitivity to cold. Pharmacological evidence shows that ginseng improves blood circulation and accelerates both metabolism and digestion. In the Japanese Pharmacopoeia Fourteenth Edition (2001), two kinds of Ginseng Radix, namely, red ginseng (steamed and dried root of *Panax ginseng*) and white ginseng (peeled and dried root of *P. ginseng*, without steaming) are described. Many pharmacological and chemical studies have been carried out on these two differently processed roots. Conclusive evidence indicates that red ginseng is superior to white ginseng as the former contains numerous effective components (Matsuura *et al.*, 1984; Kitagawa *et al.*, 1983; Samukawa *et al.*, 1995) and possesses stronger physiological activity compared with the latter (Matsuda *et al.*, 1985, 1986, 1987a, 1987b; Matsuda and Kubo, 1983, 1984, 1985). Ginsenoside-Rg<sub>3</sub>, an artifact formed in the preparation of red ginseng from crude root of *P. ginseng* (Kitagawa *et al.*, 1983, 1989; Kasai *et al.*, 1983), has been reported to show inhibitory effects on blood aggregation (Yamamoto *et al.*, 1988) and on the metastasis of tumour cells (Sato *et al.*, 1994). Furthermore, it increases blood circulation (Matsuda *et al.*, 2000). In our preliminary hair growth promoting assay of several Chinese crude drugs using the ddY strain mouse model, it has been found that a 50% ethanol extract from Ginseng Radix (roots of *P. ginseng*) exhibited significant

activity (Kubo *et al.*, 1988). In order to confirm the hair growth promoting activity of Ginseng Radix and to identify the active constituents, a newer *in vitro* and/or *in vivo* hair growth evaluation system was sought.

In recent years, the study of hair growth promoting agents has greatly advanced. Several animal models, beginning with the C3H mouse (Hattori and Ogawa, 1983) have been used experimentally to evaluate the extent of hair growth. Newer techniques for evaluation based on cell cultures, such as dermal papillae cells, hair matrix cells and outer root sheath cells (ORSCs) have been developed to analyse quantitatively the extent of hair growth *in vitro* (Messenger, 1984; Arase *et al.*, 1991; Fujie *et al.*, 1993). Among the cells that constitute hair tissue it is possible to perform a successive culture of dermal papillae cells and ORSCs, however, this has not yet been confirmed in the case of matrix cells. Because of the complicated structure of hair tissue, it is presumed that even if epithelial or mesenchymal cells could be cultivated separately clear results about hair growth would not be forthcoming.

More recently, organ culture methods of hair follicles have been developed to evaluate quantitatively the extent of hair growth *in vitro* (Jindo and Tsuboi, 1997; Imai *et al.*, 1993; Jindo *et al.*, 1993; Philpott *et al.*, 1990). The organ culture method separately cultivates each individual hair follicle. This evaluation system is thought to be correlated with *in vivo* systems because the extent of hair growth can be observed as the sum of the functions of each cell (Jindo and Tsuboi, 1997). Thus, the activity of two kinds of Ginseng Radix and major ginseng saponins were examined using the organ culture of vibrissal hair follicles. This report deals with the hair growth promoting effect of Ginseng Radix on vibrissal hair follicles (excised from the upper lip region of mice) in order to explore natural resources involved in the acceleration and promotion of hair growth.

\* Correspondence to: Dr H. Matsuda, Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashiosaka, Osaka, 577-8502, Japan. E-mail: matsuda@phar.kindai.ac.jp

## MATERIALS AND METHODS

**Plant materials.** Red ginseng and white ginseng originating from the roots of *P. ginseng*, cultivated in Korea, were provided by Japan Korea Red Ginseng Co., Ltd.

**Preparation of 70% methanol extract from Red Ginseng or White Ginseng and ginsenosides.** The crushed roots of red ginseng or white ginseng were extracted twice with 70% methanol, under reflux, for 2 h. These extracts were evaporated under reduced pressure and then lyophilized to give two 70% methanol extracts (RG-ext, yield: 32.0%, WG-ext, yield: 19.9%). G-Rb<sub>1</sub>, G-Rg<sub>1</sub>, G-Ro and 20(S)-G-Rg<sub>3</sub> were isolated from red ginseng by the method previously described (Kitagawa *et al.*, 1983, 1989). The ginsenoside content in these extracts was determined using the high performance liquid chromatography (HPLC) method as described by Samukawa *et al.* (1995). The HPLC conditions were as follows: apparatus, Shimadzu LC-6A system equipped with a Shimadzu SPD-6A detector, Shimadzu SLC-6A system controller, Shimadzu CTO-6A column oven and Shimadzu chromatopac C-R3A; column, Superspher RP-18(e) (4.0 i.d. × 250 mm, Merck); eluent, (A) CH<sub>3</sub>CN–H<sub>2</sub>O–0.1% H<sub>3</sub>PO<sub>4</sub> (21:72:8 v/v), (B) CH<sub>3</sub>CN, flow rate 0.8 mL/min [linear gradient flow programme: (A) 0–19 min; 100%, 19–20 min; 100%–90%, 20–73 min 90%, 73–103 min; 90%–70%, 103–130 min; 70%]; column temperature: programme (0–30 min; 35 °C, 30–60 min; 55 °C, 60–130 min; 35 °C; detection, UV 202 nm; retention time (min), G-Rb<sub>1</sub>; 68, G-Rg<sub>1</sub>, 27, G-Ro; 72 and 20(S)-G-Rg<sub>3</sub>; 123. The results are shown in Table 3.

**Animals.** B6C3F<sub>1</sub> mice were used for all experimental procedures. Three to eight-day-old pups with their mothers were purchased from Japan SLC (Shizuoka, Japan). The mice were maintained in an air-conditioned room with light from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chows (Labo MR Stock, Nihon Nosan Kogyo) and water were given freely. Nine-day-old mice were used for experiments.

**Organ culture of mouse vibrissal hair follicles.** Hair growth activity using an organ culture of mouse vibrissal hair follicles was performed according to the method described by Jindo and Tsuboi (1997) with minor modifications. Under the stereomicroscope, normal anagen vibrissal hair follicles were obtained from these mice under ether anaesthesia using a scalpel and tweezers. The vibrissal hair follicles were removed from the upper lip region. A total of nine intact follicles from the two ventral and dorsal rows nearest the eye were harvested from each pad. Follicles from each litter of pups were pooled. Isolated vibrissal hair follicles of mice were placed in RPMI 1640 medium (Gibco™, Invitrogen Corp., CA USA) containing 50 unit/mL penicillin and 50 µg/mL streptomycin (penicillin–streptomycin, Gibco™, Invitrogen Corp.). After washing with RPMI 1640 medium, the vibrissal hair follicles were cultured in RPMI 1640 medium at 31 °C in 95% O<sub>2</sub>–5% CO<sub>2</sub>. Each organ culture dish (Falcon 3037, Becton-Dickinson Labware, Franklin Lakes, NJ, USA) contained 0.75 mL of the medium in the centre well, over which a stainless

steel mesh and lens paper were placed. Three vibrissal hair follicles were placed on the lens paper in each dish and immersed into the medium so as to be covered with a thin layer of medium. Each group consisted of four dishes. After pre-incubation for 12 h, the medium was exchanged for one containing each sample solution. The samples dissolved with 50% ethanol were added to the medium (final concentration: samples; 1–50 µg/mL, ethanol; 0.2% (v/v)). The medium was changed during the culture period (72 h) every 24 h. Human hepatocyte growth factor/scatter factor (HGF/SF, R&D Systems Inc., MN, USA) was used as a positive control (Jindo and Tsuboi, 1997).

**Measurement of length of vibrissal hair follicle.** The individual hair follicles were photographed immediately prior to and 48 h and 72 h after the start of incubation (magnification × 20). Changes in hair length were calculated from the photographs and expressed as mean ± SE of 11–12 vibrissal hair follicles.

**Statistical analysis.** The experimental data were tested for statistical significance using Bonferroni/Dunn's multiple range test method.

## RESULTS AND DISCUSSION

As shown in Table 1, RG-ext promoted hair growth in a dose-dependent manner after both 48 h and 72 h of culture. It exhibited its most significant effect at a dosage of 50 µg/mL. The effect of WG-ext was lower compared with that of RG-ext. These results suggest that ginsenosides play a major role in Ginseng Radix mediated hair growth promotion since they are found more abundantly in RG-ext compared with WG-ext.

To date, 26 saponins have been isolated and identified in Ginseng Radix. These saponins are divided broadly into three groups termed panaxadiols, panaxatriols and oleananes based on the basic structure of aglycones produced by hydrolysis. Among these saponins, G-Rb<sub>1</sub> in the panaxadiol saponins, G-Rg<sub>1</sub> in the panaxatriol saponins and G-Ro (a peculiar component in Ginseng Radix) in the oleanane saponins are relatively abundant in Ginseng Radix. In order to clarify the active ingredient, the hair growth promoting effects of these three saponins were investigated.

As shown in Table 2, G-Rb<sub>1</sub> promoted hair growth in a dose-dependent manner, with the most significant effect seen at 10 µg/mL after 48 h culture. G-Rg<sub>1</sub> and G-Ro, however, did not exhibit any similar effects. It has been reported that G-Rb<sub>1</sub> can promote nerve fibre growth (Saito *et al.*, 1977), protein synthesis (Shibata *et al.*, 1976) and RNA polymerase activity (Iijima *et al.*, 1976). In the organ culture system of mouse vibrissal hair follicles, it can be assumed that G-Rb<sub>1</sub> acts as an activator for promoting hair growth at a cellular level. From the results in Table 3, it seems quite probable that Rb<sub>1</sub> is one of active ingredients because it is present in relatively large quantities in RG-ext.

Some of the panaxadiol saponins (including ginsenoside-Rb<sub>1</sub>, -Rb<sub>2</sub>, -Rc and -Rd) are known to be converted to 20(S)- and 20(R)-G-Rg<sub>3</sub> during the processing of the crude root of *P. ginseng* to red ginseng (Kitagawa *et al.*, 1983, 1989; Kasai *et al.*, 1983).

**Table 1. Hair growth effect of 70% methanol extract from red ginseng (RG-ext) or white ginseng (WG-ext) on organ culture of mouse vibrissal hair follicles**

Sample	Conc. ( $\mu\text{g/mL}$ )	After 48 h		After 72 h	
		Length ( $\mu\text{m}$ )	Activation (%)	Length ( $\mu\text{m}$ )	Activation (%)
Control	–	476.1 $\pm$ 34.8	–	625.9 $\pm$ 48.7	–
RG-ext	20	522.2 $\pm$ 26.1	9.7	663.0 $\pm$ 31.1	5.9
	50	559.3 $\pm$ 21.8 <sup>a</sup>	17.5	746.3 $\pm$ 29.5*	19.2
Control	–	574.9 $\pm$ 32.2	–	728.4 $\pm$ 55.1	–
WG-ext	20	539.7 $\pm$ 38.3	Non active	732.2 $\pm$ 62.3	4.1
	50	628.4 $\pm$ 47.3	9.2	885.7 $\pm$ 51.5*	21.1

Individual vibrissal hair follicles from B6C3F<sub>1</sub> mouse were micro-dissected and cultured in RPMI 1640 medium at 31 °C in 95% O<sub>2</sub>–5% CO<sub>2</sub>. After pre-incubation for 12 h, medium was exchanged for medium containing sample solutions. The individual hair follicles were photographed immediately prior to 48 h and 72 h after the start of incubation. Changes in hair length were calculated from the photographs and reported as mean  $\pm$  SE of 11–12 vibrissal hair follicles. Significantly different from the control group. <sup>a</sup>  $p < 0.05$ .

**Table 2. Hair growth effects of ginsenoside-Rb<sub>1</sub> (G-Rb<sub>1</sub>), -Rg<sub>1</sub> (G-Rg<sub>1</sub>) and -Ro (G-Ro) on organ culture of mouse vibrissal hair follicles**

Sample	Conc. ( $\mu\text{g/mL}$ )	After 48 h		After 72 h	
		Length ( $\mu\text{m}$ )	Activation (%)	Length ( $\mu\text{m}$ )	Activation (%)
Control	–	574.9 $\pm$ 32.2	–	728.4 $\pm$ 55.1	–
G-Rb <sub>1</sub>	1	579.8 $\pm$ 26.2	0.9	749.0 $\pm$ 33.9	2.7
	10	665.2 $\pm$ 13.2 <sup>a</sup>	15.7	847.4 $\pm$ 22.7	16.2
Control	–	476.1 $\pm$ 34.8	–	625.9 $\pm$ 48.7	–
G-Rg <sub>1</sub>	10	508.5 $\pm$ 23.7	6.8	608.5 $\pm$ 33.8	Non active
Control	–	476.1 $\pm$ 34.8	–	625.9 $\pm$ 48.7	–
G-Ro	10	488.1 $\pm$ 35.7	2.5	634.0 $\pm$ 52.9	1.3

According to the method in Table 1. Each value represents the mean  $\pm$  SE of 11–12 vibrissal hair follicles. Significantly different from the control group. <sup>a</sup>  $p < 0.05$ .

**Table 3. Ginsenoside contents in red ginseng (RG-ext) or white ginseng (WG-ext)**

Ginsenoside	Content (mg/g, crude drug)	
	RG-ext	WG-ext
G-Rb <sub>1</sub>	5.98	2.32
G-Rg <sub>1</sub>	2.96	1.35
G-Ro	1.60	0.68
20(S)-G-Rg <sub>3</sub>	0.20	0.01

Values are average calculated from three measurements of each ginseng ( $n = 3$ ).

**Table 4. Hair growth effect of 20(S)-ginsenoside-Rg<sub>3</sub> (20(S)-G-Rg<sub>3</sub>) and human hepatocyte growth factor/scatter factor (HGF/SF) on organ culture of mouse vibrissal hair follicles**

Sample	Conc. ( $\mu\text{g/mL}$ )	After 48 h		After 72 h	
		Length ( $\mu\text{m}$ )	Activation (%)	Length ( $\mu\text{m}$ )	Activation (%)
Control	–	613.8 $\pm$ 12.6	–	818.1 $\pm$ 20.2	–
20(S)-G-Rg <sub>3</sub>	1	597.0 $\pm$ 28.1	0.9	830.3 $\pm$ 37.7	1.5
	10	695.6 $\pm$ 30.2 <sup>a</sup>	13.4	880.5 $\pm$ 40.1	7.5
HGF/SF	20(ng/mL)	665.6 $\pm$ 20.3 <sup>a</sup>	8.4	853.6 $\pm$ 21.4	4.2

According to the method in Table 1. Each value represents the mean  $\pm$  SE of 11–12 vibrissal hair follicles. Significantly different from the control group. <sup>a</sup>  $p < 0.01$ .

20(S)-G-Rg<sub>3</sub> significantly promoted hair growth at a dosage of 10  $\mu\text{g/mL}$  after 48 h of culture (Table 4). As shown in Table 4, a positive control, HGF/SF, showed 8.4% and 4.2% activation after 48 and 72 h, respectively.

## CONCLUSIONS

Commercially available hair care agents include anti-inflammatory, antiandrogenic and antibacterial agents

along with vitamins and moisturizers. Ginseng Radix extracts are also found in such hair care agents. However, no detailed explanations are given about the mechanism of hair growth or the major active constituents. Increased blood circulation around the head dermis may provide hair roots with addi-

tional energy and thereby stimulate hair growth. The results presented here indicated that Ginseng Radix possesses hair growth promoting activity and that G-Rb<sub>1</sub> may be one of the active constituents of Ginseng Radix in the mouse vibrissal hair follicle organ culture model.

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