

The hair growth promoting effect of *Sophora flavescens* extract and its molecular regulation

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Received 24 January 2002; received in revised form 8 May 2002; accepted 9 May 2002

Abstract

In search of natural extracts for hair growth, we found that the extract of dried root of *Sophora flavescens* has outstanding hair growth promoting effect. After topical application of *Sophora flavescens* extract onto the back of C57BL/6 mice, the earlier conversion of telogen-to-anagen was induced. The growth of dermal papilla cells cultured in vitro, however, was not affected by *Sophora flavescens* extract treatment. RT-PCR analysis showed that *Sophora flavescens* extract induced mRNA levels of growth factors such as IGF-1 and KGF in dermal papilla cells, suggesting that the effects of *Sophora flavescens* extract on hair growth may be mediated through the regulation of growth factors in dermal papilla cells. In addition, the *Sophora flavescens* extract revealed to possess potent inhibitory effect on the type II 5 α -reductase activity. Taken together, these results suggest that *Sophora flavescens* extract has hair growth promoting potential and can be used for hair growing products. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Sophora flavescens*; Hair growth; Growth factors; Type II 5 α -reductase

1. Introduction

Hair growth is a complex and cyclically controlled process that is characterized by a finite period of hair fiber production (anagen), a brief regression phase (catagen), and a resting period (telogen) [1–3]. Although the precise mechanism

regulating the hair growth cycle has not yet been fully understood, several factors were implicated to exert their specified roles in hair growth control. Androgens are well known to cause regression and balding on the scalp in genetically disposed individuals. Testosterone and dihydrotestosterone (DHT), which is formed from testosterone by the action of 5 α -reductase, are two major androgens and DHT has been considered more potent to trigger hair growth or hair loss [4,5]. There are two types of 5 α -reductase, type I and type II. Although type I is predominant in scalp, type II has been

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identified to have a crucial role in the hair growth regulation. The important role of type II 5 α -reductase is supported by a well-documented male pseudohermaphroditism where type II 5 α -reductase is deficient and shows no androgenetic alopecia [6,7]. And finasteride (Merck), the inhibitor of type II 5 α -reductase, was approved for use in men with androgenetic alopecia by the Food and Drug Administration (FDA).

Androgens affect the dermal papilla (DP) of hair follicle, which produces paracrine signals that stimulate or inhibit the growth of follicular epithelium. These include insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) [8,9]. In addition, other growth factors are also found to be involved in the hair growth regulation. For examples, keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) have a stimulatory effect on hair follicle growth, while epidermal growth factor (EGF) and transforming growth factor- β (TGF- β) have an inhibitory effect on hair follicle growth [10–15].

Many plant extracts have been traditionally used for treating hair loss in oriental medicine. We examined 45 different kinds of plant extracts and discovered that *Sophora flavescens* extract has the best hair growth promoting effect. We report here that *Sophora flavescens* extract stimulates the telogen to anagen transformation in C57BL/6 mice and upregulates the mRNA expression of IGF-1 and KGF in human dermal papilla cells. And *Sophora flavescens* extract also has the type II 5 α -reductase inhibiting activity.

2. Materials and methods

2.1. Materials

The dried root of *Sophora flavescens* was purchased from Kyeonghui (Geumsan, Chungnam, Korea). C57BL/6 mice were supplied from Dae-Han Biolink (Eumsung, Chungbuk, Korea). Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) came from Gibco BRL (Gaithersburg, MD), [methyl-³H]thymidine (40–60 Ci/mmol) and [1,2,6,7-³H]testosterone (70–105 Ci/

mmol) from Amersham Pharmacia Biotech (Buckinghamshire, UK). M-MLV reverse transcriptase and Taq polymerase were purchased from Promega (Madison, WI).

2.2. Preparation of *Sophora flavescens* extract

The dried root of *Sophora flavescens* was crushed and extracted with cold methanol. The methanol-extract was concentrated in a vacuum evaporator (Büchi, Switzerland) and resulting residue was weighed and dissolved to 1% solution in 50% ethanol.

2.3. Animal test

Six-week-old female C57BL/6 mice were obtained and then allowed to adapt for 1 week with food and water ad libitum. The backs of mice were shaved with animal clipper at 7 weeks of age, at which all follicles were synchronously in telogen stage. From the following day (day 1), 0.2 ml of 1% *Sophora flavescens* extract in 50% ethanol was topically applied daily for 30 days. Hair growth promoting effect was easily checked by the darkening of skin color, which indicated telogen-to-anagen conversion [16].

2.4. Isolation and culture of human hair dermal papilla cells

Human anagen hair follicles were isolated from scalp skin of normal men aged from 25 to 30 undergoing plastic surgery. The dermal papilla (DP) cells were isolated and cultured as previously described [17] with slight modifications. DP cells were maintained in DMEM supplemented with 10% FCS at 37 °C in an atmosphere of 5% CO₂. The second or third passage DP cells were used in this study.

2.5. [³H]thymidine incorporation

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 μ Ci of [³H]thymidine and *Sophora flavescens* extract as

indicated concentrations. Following incubation for 48 h, cells were washed twice with PBS, once with 5% cold TCA. Cells were then lysed with 0.1 N NaOH, 1% SDS and the radioactivity was measured by liquid scintillation counter (Beckman).

2.6. 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 *Sophora flavescens* extract as indicated concentrations for 18 h. Total RNAs were extracted by acid guanidinium thiocyanate–phenol–chloroform method [18]. Two µg of total RNAs were reverse transcribed with M-MLV reverse transcriptase (Promega) in the presence of random hexamer. The resultant RT mixtures were then subjected to PCR cycles as follows: 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min for 40 cycles (IGF-1, HGF and VEGF) and 35 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).

2.7. 5 α -reductase assay

Type II 5 α -reductase assay was carried out according to previously described method [19]. Briefly, the prostate was dissected from male Sprague–Dawley rats and homogenized in a solu-

tion containing 40 mM KH₂PO₄, pH 6.5, 5 mM MgSO₄, 25 mM KCl, 1 mM PMSF, 1 mM DTT and 250 mM sucrose. After centrifugation at 1500 \times g for 15 min, crude nuclear fraction was resuspended in 40 mM sodium citrate, pH 5.5, 1 mM DTT, 1 mM NADPH, 100 nM [³H]testosterone, and *Sophora flavescens* extract was added as indicated concentrations. Reaction mixtures were incubated at 37 °C for 1 h, followed by steroids extraction with 70% cyclohexane, 30% ethyl acetate. Solvent was dried and steroids were reconstituted with chloroform, spotted onto TLC plate and developed in 80% toluene, 20% acetone. After autoradiography, 5 α -reductase activities were measured by a densitometer (Imagemaster, Pharmacia Biotech).

2.8. Statistical analysis

Data for RT-PCR were statistically evaluated using Student's *t*-test. Statistical significance was set at *P* < 0.05.

3. Results

3.1. Hair growth promoting effect of *Sophora flavescens* extract

In an attempt to search for hair growth promoting materials from alternative medicine, we have performed massive screening of plant extracts using C57BL/6 mice model. Among all the plant extracts, *Sophora flavescens* extract showed outstanding hair growth promoting potential. After

Table 1
Nucleotide sequence of the primers and expected size of PCR products

Growth factor	Primer	Expected Size (bp)
IGF-1	Forward (5' → 3')	TCAACAAGCCCACAGGGTAT
	Reverse (5' → 3')	ACTCGTGCCAGAGCAAAGGAT
HGF	Forward (5' → 3')	CGAGGCCATGGTGCTATACT
	Reverse (5' → 3')	ACACCAGGGTGATTCAGACC
KGF	Forward (5' → 3')	GACATGGATCCTGCCAACTT
	Reverse (5' → 3')	AATCCAAGTCCCACTGTCC
VEGF	Forward (5' → 3')	TCTCAAGCCATCCTGTGTG
	Reverse (5' → 3')	GCGAGTCTGTGTTTTTGCAG

topical application onto the back skins of C57BL/6 mice daily up to 30 days, *Sophora flavescens* extract induced earlier telogen-to-anagen conversion as compared to vehicle treated group (Fig. 1A, B). Histologic studies showed that *Sophora flavescens* extract markedly increased the depth and size of hair follicles as compared with control group (Fig. 1C, D). This result clearly supports that *Sophora flavescens* extract induces early onset of anagen and stimulates hair growth.

3.2. Effects of *Sophora flavescens* extract on the cultured dermal papilla cells

Recently, Takahashi et al. [20] reported that *Sophora flavescens* extract has a potential to elongate anagen hair follicle and to stimulate the growth of outer root sheath cells cultured in vitro. To investigate whether *Sophora flavescens* extract has a mitogenic effect, we adopted human hair dermal papilla (DP) cell culture system. When *Sophora flavescens* extract was added into growth medium, however, there was no obvious growth stimulating effect on the DP cells (Table 2). This

Table 2

Effect of *Sophora flavescens* extract on [³H]thymidine incorporation into cultured dermal papilla cells

Group	Incorporation ratio (%)
Control	100±2.1
<i>Sophora flavescens</i> extract, 0.001%	85.2±3.5
<i>Sophora flavescens</i> extract, 0.0001%	98.0±2.6
<i>Sophora flavescens</i> extract, 0.00001%	99.7±2.0

result suggests that the hair growth promoting effect of *Sophora flavescens* extract may be mediated through another pathway rather than the mitogenic effect on the DP cells. Thus, we decided to investigate the effect of *Sophora flavescens* extract on the expression of several growth factors that were implicated in the regulation of hair growth. To this end, we performed semi-quantitative RT-PCR analysis. As shown in Fig. 2, *Sophora flavescens* extract induced mRNA levels of IGF-1 and KGF dose-dependently in the DP cells. The mRNA levels of HGF and VEGF, however, were not affected by *Sophora flavescens* extract treatment.

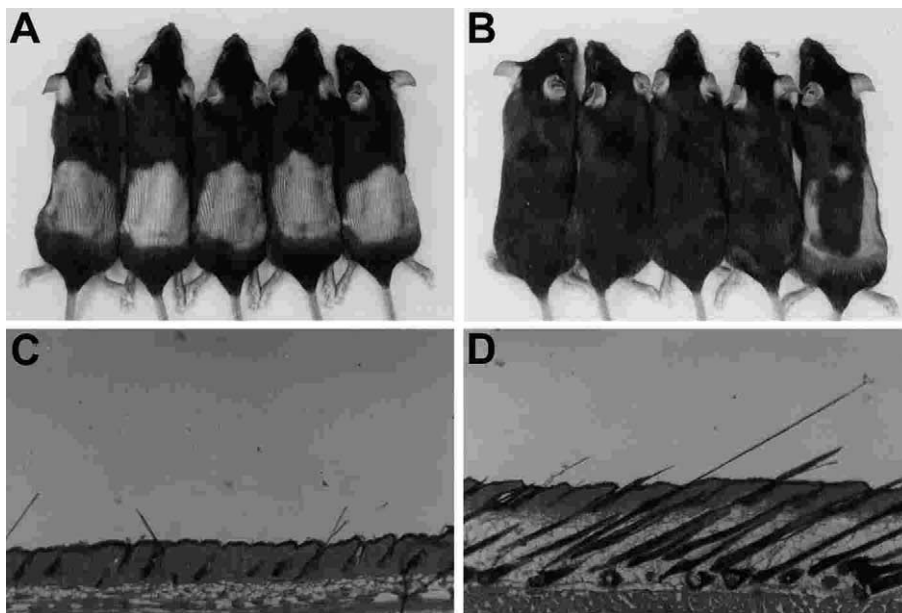


Fig. 1. Hair growth promoting effect of *Sophora flavescens* extract in C57BL/6 mice. The back skins of C57BL/6 mice were shaved, and then *Sophora flavescens* extract was topically applied for 30 days. (A) Control, 50% ethanol, (B) 1% *Sophora flavescens* extract in 50% ethanol. Low panel shows representative histological data of back skin of control (C) and *Sophora flavescens* treated mouse (D).

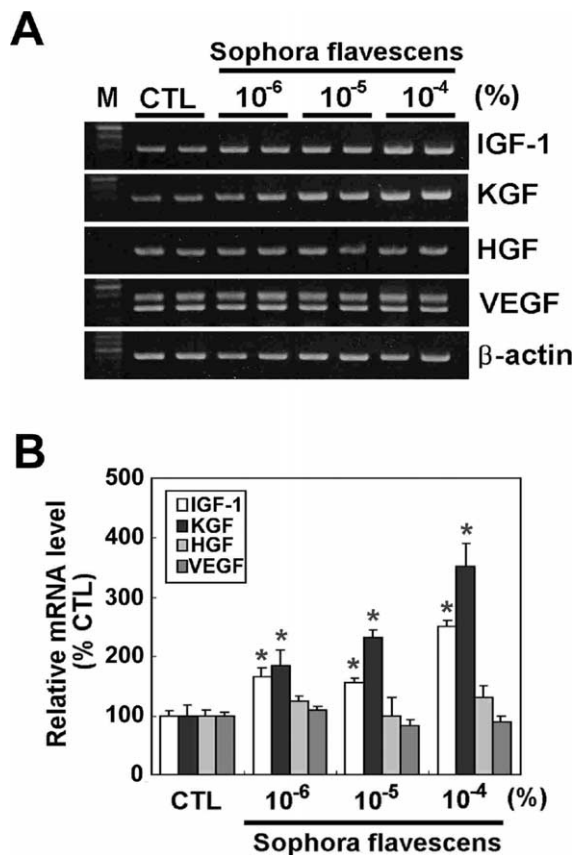


Fig. 2. (A) Effect of *Sophora flavescens* extract on the mRNA level of growth factors in cultured dermal papilla cells by semi-quantitative RT-PCR analysis. (B) Quantification of RT-PCR products for growth factors using a densitometer. The amount of RT-PCR products for each growth factors was corrected according to the quantity of β -actin and expressed as a percentage of control (mean \pm S.E., $P < 0.05$).

3.3. Inhibitory effect of *Sophora flavescens* extract on the type II 5α -reductase activity

It has been documented that *Sophora flavescens* extract has anti-androgen effect [21]. We also previously reported that *Sophora flavescens* extract had a potential for inhibiting the type I 5α -reductase activity [22]. These previous findings led us to examine the effect of *Sophora flavescens* extract on the type II 5α -reductase activity. Using rat prostate as a source for type II 5α -reductase, we evaluated the effect of *Sophora flavescens* extract. As shown in Fig. 3, *Sophora flavescens*

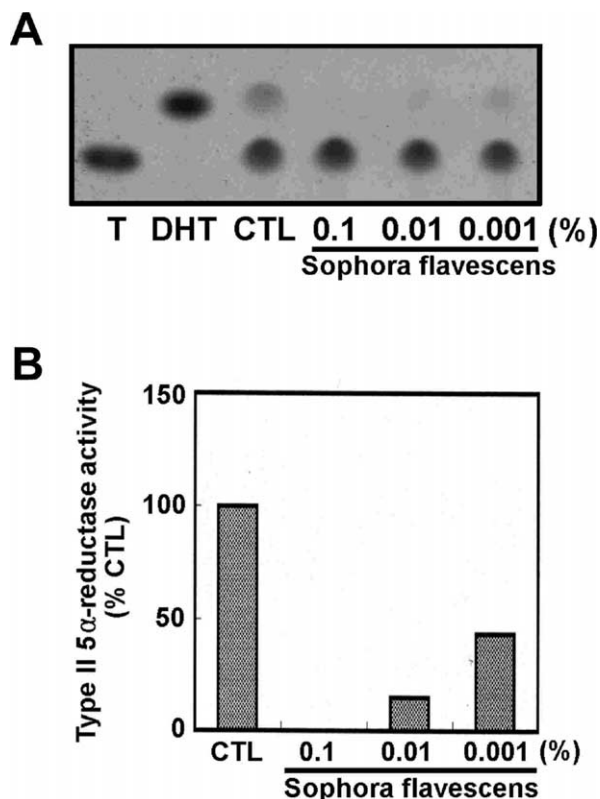


Fig. 3. Representative autoradiogram of type II 5α -reductase assay (A). Type II 5α -reductase activities were measured using a prostate of Sprague–Dawley rat. After incubation, steroids were extracted, separated on TLC plate, and then exposed to Hyperfilm. T, testosterone; DHT, dihydrotestosterone; CTL, control; 0.1, *Sophora flavescens* extract, 0.1%; 0.01, *Sophora flavescens* extract, 0.01%; 0.001, *Sophora flavescens* extract, 0.001%. (B) Quantification of type II 5α -reductase activities. Conversion ratios of T to DHT were calculated using a densitometer and expressed as a percentage of control.

extract inhibited the type II 5α -reductase activity dose-dependently.

4. Discussion

Recently, the number of men and women who suffered from hair loss and/or hair thinning is increasing. Thus it is very important to develop new therapeutic materials to stop hair loss and to enhance hair growth. Alternative medicine is one interesting area, which is getting more popular. Although it has not yet been incorporated into the

mainstream of medical care because of limited scientific evidences and lack of mechanistic understanding, alternative medicine is becoming an increasingly attractive approach all over the world [23]. *Sophora flavescens*, one of long-been used medicinal plants in oriental medicine, was well known to have a variety of activities against many diseases. These include anti-bacterial, anti-ulceral and vasodilator activities [21,24,25]. Furthermore, it has been also reported that *Sophora flavescens* extract has an apoptogenic effect and an inhibitory role in phospholipase C pathway [26,27].

In the present study, we have demonstrated that *Sophora flavescens* extract has an outstanding hair growth promoting effect. In addition, *Sophora flavescens* extract showed the regulatory role on the expression of growth factors and the inhibitory effect on type II 5 α -reductase.

The mesenchyme-derived DP cells are well characterized to have a regulatory role in hair growth, and it is believed that diffusible factors like IGF-1 and KGF from the DP could directly affect follicular epithelium [1,8]. It has been previously documented that IGF-1 could stimulate the growth of epithelial cells as well as hair follicle tissue cultured in vitro [28]. Moreover, in IGF-1 transgenic animals, hair elongation was significantly increased compared to their littermates [29]. KGF, which belongs to the member of the fibroblast growth factor (FGF) family, is an important paracrine mediator of proliferation and differentiation in a wide variety of epithelial cells [30]. The regulatory role of KGF in hair growth is well implicated in transgenic mice model in which KGF directly affects the development of hair follicles [10]. Accordingly, it is regarded that the regulation of growth factors like IGF-1 and KGF in the DP cells is fundamental to the growth control of hair follicle. In this study, we have shown that *Sophora flavescens* extract induced IGF-1 and KGF mRNA levels in the DP cells. These results suggest that *Sophora flavescens* extract may produce positive effects on the hair growth promotion partly through the regulation of IGF-1 and KGF in the DP cells.

The important role of 5 α -reductase in the hair growth regulation is well recognized. It has been

previously reported that metabolism of testosterone in hair follicles is different within body sites depending on their androgen sensitivity, and the type II 5 α -reductase in the DP plays a central role by the intrafollicular conversion of testosterone to dihydrotestosterone [5]. Thus, type II 5 α -reductase is considered to be one of the most important targets for developing drugs for treatment of hair loss. In this regard, it is noteworthy that *Sophora flavescens* extract has a potent inhibitory effect on the type II 5 α -reductase activity.

Overall, we have demonstrated the potent hair growth promoting effect and the putative molecular regulatory roles of *Sophora flavescens* extract, suggesting that *Sophora flavescens* extract may be a good candidate for helping hair growth promotion.

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