Ginsenoside F2 Reduces Hair Loss by Controlling Apoptosis through the Sterol Regulatory Element-Binding Protein Cleavage Activating Protein and Transforming Growth Factor-β Pathways in a Dihydrotestosterone-Induced Mouse Model

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This study was conducted to test whether ginsenoside F2 can reduce hair loss by influencing sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP) and the transforming growth factor beta (TGF-β) pathway of apoptosis in dihydrotestosterone (DHT)-treated hair cells and in a DHT-induced hair loss model in mice. Results for ginsenoside F2 were compared with finasteride. DHT inhibits proliferation of hair cells and induces androgenetic alopecia and was shown to activate an apoptosis signal pathway both in vitro and in vivo. The cell-based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed that the proliferation rates of DHT-treated human hair dermal papilla cells (HHDPs) and HaCaTs increased by 48% in the ginsenoside F2-treated group and by 12% in the finasteride-treated group. Western blot analysis showed that ginsenoside F2 decreased expression of TGF-β2 related factors involved in hair loss. The present study suggested a hair loss related pathway by changing SCAP related apoptosis pathway, which has been known to control cholesterol metabolism. SCAP, sterol regulatory element-binding protein (SREBP) and caspase-12 expression in the ginsenoside F2-treated group were decreased compared to the DHT and finasteride-treated group. C57BL/6 mice were also prepared by injection with DHT and then treated with ginsenoside F2 or finasteride. Hair growth rate, density, thickness measurements and tissue histotological analysis in these groups suggested that ginsenoside F2 suppressed hair cell apoptosis and premature entry to catagen more effectively than finasteride. Our results indicated that ginsenoside F2 decreased the expression of TGF-β2 and SCAP proteins, which have been suggested to be involved in apoptosis and entry into catagen. This study provides evidence those factors in the SCAP pathway could be targets for hair loss prevention drugs.

Key words ginsenoside F2; dihydrotestosterone; apoptosis; finasteride

Hair follicle repeats a cyclical process consisting of three phases: a growing phase (anagen), a regressing phase (catagen), and a resting phase (telogen). During hair loss, the hair growth cycle shows multiple abnormalities including curtailed anagen and premature catagen, and vellus hair follicles show conversion into terminal hair follicles.

Androgenetic alopecia (AGA) is the most common type of hair loss. Increased conversion from testosterone to dihydrotestosterone (DHT), potentiated by increased androgen receptor (AR) in the hair follicle, and is seen in AGA. Finasteride, a proprietary pharmaceutical, is often used for treatment of AGA. Although finasteride, a synthetic type II 5α-reductase inhibitor, reduces the conversion of testosterone to DHT. Also, finasteride suppressed caspase 1, 3, 8 and 9 expression. But, it has adverse sexual side effects. We wanted to find natural plant components having less severe side effects that could be used instead of finasteride.

DHT induces hair follicles to enter the catagen phase associated with apoptosis by increasing levels of transforming growth factor (TGF)-β2. TGF-β2 not only inhibits hair growth by reducing anagen and promoting early entry into catagen, but also affects the expression of smad-2 and -3. These factors influence hair cell apoptosis by direct regulation of Bcl-xL and caspase-3. Binding of DHT to the AR influences specificity protein 1 (sp1), and then controls the expression of TGF-β2. Despite research concerning the roles of sp1 and TGF-β2 in tumorigenesis, their roles in hair loss have not yet been studied. This led us to investigate the relationships between DHT, TGF-β2 and transcription factor sp1 and their influence on hair loss.

Studies on the mechanisms of apoptosis in hair follicles have focused on TGF-β signaling. However, there have been no studies on other mechanisms of apoptosis at work in the hair cycle. This led us to investigate the sterol regulatory element-binding protein cleavage-activating protein (SCAP), which is known to be affected by the binding of DHT to the AR. SCAP is a factor involved in cholesterol metabolism. Sterol regulatory element-binding protein (SREBP) exists on the nuclear membrane and endoplasmic reticulum (ER). It moves to the Golgi body and induces ER stress which causes activation of caspase-3 and 12, and eventually induces apoptosis. Because apoptosis of cells in the hair follicle is thought to play a major role in the process of hair loss, it may be useful to study the relationships between SCAP, TGF-β2 and the caspases.

Panax ginseng is used as a medicinal plant in some traditions and its main physiologically active compounds are the ginsenosides. A major ginsenoside Rb1, is hydrolyzed by intestinal microorganisms in the body into the minor ginsenoside F2. Ginsenoside F2 has been the focus of some inves-
Ginsenoside Rb1, precursor of ginsenoside F2, has been reported to prevent apoptosis induced by external stimuli in human keratinocyte cells (HaCaTs) as well as to inhibit activation by DHT. However, the effects of ginsenoside F2 on hair loss have not been reported.

The aim of this study was to investigate the effects of ginsenoside F2 on AGA and evaluate its impact on SCAP, TGF-β/2 and other factors in this apoptosis pathway. Experiments were conducted using a DHT-induced hair loss model in dermal papilla cells in vitro and C57BL/6 mice in vivo, and results obtained with ginsenoside F2 were compared to those obtained with finasteride.

**MATERIALS AND METHODS**

**Materials** The mesenchymal stem cell (MSC) medium kit came from Sciencell (Carlsbad, U.S.A.). Fetal bovine serum (FBS) was obtained from Gibco-BRL (Gaithersburg, MD, U.S.A.). RPMI-1640, antibiotics and trypsin were purchased from Cambrex (Walkersville, MD, U.S.A.). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), radio immunoprecipitation assay buffer (RIPA buffer), carboxymethylcellulose (CMC), dimethyl sulfoxide (DMSO), DHT, finasteride, Sepharose, hematoxylin and eosin stain and reagents without indication were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Immobilon-P transfer membranes were purchased from Millipore Corporation (Bedford, MA, U.S.A.). Enhanced chemiluminescence (ECL) Western blotting detection system and lysis buffer for cells and protein in skin were purchased from Intron (Sungnam, Korea). The antibodies primary and secondary antibodies conjugated to horseradish peroxidase were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

**Preparation of Ginsenoside F2** Ginsenoside Rbl was converted to ginsenoside F2 by incubation with Aspergillus niger that was cultured in tryptic soy broth supplemented with ginsenoside Rbl for 7 d at 37°C with agitation. The progress of conversion was monitored by TLC once a day. After a satisfactory extent of conversion was obtained, the medium was extracted with butanol. The supernatant was fractionated and concentrated with a vacuum rotary evaporator.

The ginsenoside F2 was analyzed by LC/MS (Waters 2795 separation module with PDA detector and Waters SQD mass). For the analysis, a Cosmosil C18 column (250×4mm, 5µm, U.S.A.) was used. Injection column was 10µL. Solvent A was water (contain 0.1% formic acid); solvent B was acetonitrile (contain 0.1% formic acid). The flow rate was 0.6mL/min. The elution program was as follows: initial 5% b, 30-100% b, 35-5% b, 45-5% b (end).

The column effluent was introduced into the mass spectrometer using electrospray ionization (ESI) in the negative ion modes. Nitrogen was used as nebulizer and curtain gas. The settings of instrumentation were: capillary voltage, 4.0kV; cone voltage, 60V; source temperature, 150°C; desolvation temperature, 350°C; dry gas 750L/h; cone gas 150L/h. As a result, purity represented 96.1%.

**Prevention of DHT-Induced Apoptosis** Cell Culture: IHDPDCs were purchased from Sciencell (Carlsbad, U.S.A.) and HaCaT cells were purchased from Korea cell line bank (Seoul, Korea). IHDPDCs were maintained with MSC medium containing 5% FBS and 1% antibiotic. HaCaT cells were incubated in RPMI-1640 containing 10% FBS and 1% antibiotic at 37°C, 5% CO2. Samples were sub-cultured every 3 d.

**MTT Assay:** After the IHDPDCs and HaCaT cells had been seeded to 5×10^3 cells/mL using a hemocytometer, 200µL of culture was put into each well of a 96-well plate for 24h. DHT, finasteride and F2 were prepared that dissolved in DMSO. When we used these samples were dilution to serum free media. After treatment with 0.1µM DHT, the samples were divided into an experimental group and control group depending on sample treat concentration. F2 was administered to the experimental group and the comparative control groups were treated with Finasteride 0.2µM. Samples were treated with F2 at concentrations of 0.01, 0.1, 1 and 10µM. Twenty four hours after treatment with DHT, samples were cultured for 4h and 20µL of MTT (5mg/mL) solution were added. The absorbance at 570nm was estimated using an ELISA on samples of culture media containing MTT solution, adding DMSO and solving formazan which was formed when it was cultured.

**Western Blot Analysis of Factors in DHT-Induced Apoptosis** Expression of proteins involved in preventing apoptosis and entry into telogen was analyzed by Western blot. IHDPDCs were cultured in 60mm dishes using a-hemocytometer to seed 1×10^6 cells per dish. Samples were then divided into an experimental group and a control group with varying reagent concentrations. Ginsenoside F2 was administered in experimental groups and finasteride 0.2µM was administered to the comparative control groups. Ginsenoside F2 was applied at concentrations of 0.1 and 1µM. Protein was isolated from cultured IHDPDCs by treating samples with lysis buffer for 24h. Protein was quantified using a protein assay reagent (Bio-Rad, Hercules, CA, U.S.A.). Twenty micrograms of protein from each group was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 12% acrylamide gel and transferred to a membrane using Immobilon-P transfer membranes. Protein on the membranes was detected using the ECL system to identify secondary antibodies after binding of specific primary antibodies. Luminescence on developed membranes was visualized using a MF-ChemiBIS 3.2 (DNR Bio-Imaging Systems Ltd., Jerusalem, Israel).

The Effect of Preventing Entry to Telogen and Mimicry of Androgenetic Aloppecia by Injection of DHT in C57BL/6 Mice Morphological Analysis: This study was reviewed and approved by the Kyung-Hee University, Institutional Animal Care and Use Committee (IACUC, KHUASP (GC)-10-005). Seven-week-old C57BL/6 mice in telogen were divided into groups of 10 mice and denuded at a uniform time point using animal clippers and hair removal cream (Veet, Oxy Reckitt Benckiser, Chartes, France). Then, 30µg of DHT was injected once into the neck region of each mouse. and 100µL of either ginsenoside F2 (0.5mg/kg or 2.5mg/kg) or finasteride (0.5mg/kg) resolved to 0.5% of the CMC were administered orally for 35d. Mice were anesthetized using isoflurane (Hana Pharm., Seoul, Korea) and changes in hair morphology were photographed by a digital camera (FUJIFILM, Finepix S3 pro). Digital pictures were analyzed using J software (National Institutes of Health, Bethesda, MD, U.S.A.) to estimate the area which is covered with hair in each mouse. Hair thickness and density were evaluated in the same 5 areas of 25mm² in each
mouse using the Aram Diagnosis scope (Aram Human Vision System, Sungnam, Korea).

Histological Analysis: Back skin was harvested for histological analysis on 35 d. Dorsal skins were fixed in 4% formaldehyde in PBS and then processed by paraffin block embedding. General histology was visualized by hematoxylin and eosin (H&E) staining. Stained tissue was observed and analyzed through an optical microscope (Carl Zeiss SMT AG Company, Oberkochen, Germany). The stained tissues were observed based on previously described methods.\textsuperscript{18} The number of follicles each in anagen (A), and telogen (T) were counted, and the A/T ratio was determined. The H&E stained slides were photographed using a digital photomicrograph and all of the images were cropped to a fixed area 300 pixels wide. Hair follicles were counted manually in a fixed area (0.09 mm\textsuperscript{2}). Digital photomicrographs were taken from representative areas at fixed magnifications of ×50 and ×200.

\textbf{Immunohistological Analysis of the Apoptosis Pathway Induced by DHT} Skin tissue samples for immunohistochemical analysis were collected by sacrificing animals after the 35th day. The frozen sections were first air-dried for 10 min. After air drying for 10 min the slides were washed three times for 10 min in Tris-buffered saline (TBS) and were then incubated with 10% normal goat serum in TBS for 2 h. Slides were incubated with appropriate dilutions of the different primary antibodies in TBS overnight at 4°C. After washing three times for 10 min in TBS, sections were stained for 90 min at room temperature (RT) with either goat antinouse, goat anti-rabbit immunoglobulin G (IgG) conjugated with fluorescein isothiocyanate (FITC) (1:1000 in TBS), or goat anti-rat IgG conjugated with Alexa Fluor 488 (1:1000 in TBS). Slides were then washed, counterstained with PI and treated with mounting medium.

\textbf{Statistical Analysis} The statistical analysis of data was conducted using a one-way ANOVA at the level of *$p<0.05$; **$p<0.01$; ***$p<0.001$.

\section*{RESULTS}

\textbf{The Inhibitory Effect of Ginsenoside F2 on DHT-Induced Apoptosis of Hair Follicle Cells} The effect of ginsenoside F2 on hair cell apoptosis was evaluated using the MTT assay in Human Hair Dermal Papilla Cells (HHDPCs) and Human Keratinocyte (HaCaTs).

Forty-eight percent of the DHT-treated cells in the control group were apoptotic. Near-complete inhibition of apoptosis was seen at an ginsenoside F2 concentration of 0.1 $\mu$M. Even though 30% of the cells underwent apoptosis in the finasteride treatment group used as a comparative control, this serves to highlight the significant effect of ginsenoside F2 in the HHDPCs (Fig. 1A). Moreover, 46% of the DHT-treated HaCaTs in the negative control group were apoptotic, and yet ginsenoside F2 inhibited apoptosis at 0.1 $\mu$M. These results suggest that ginsenoside F2 is effective at preventing DHT-induced hair cell apoptosis and in fact compares favorably with finasteride (Fig. 1B).

\textbf{Ginsenoside F2 Reduces DHT-Induced Apoptosis in HHDPCs} The expression of sp1 is a factor affecting the expression of TGF-β2. Sp1 expression was 58% lower in the group treated with 0.1 $\mu$M ginsenoside F2 and 79% lower in the group treated with 1 $\mu$M ginsenoside F2 when compared to the control group (Fig. 2A). The expression of TGF-β2 was 51% and 72% lower than the control in the groups treated with 0.1 $\mu$M and 1 $\mu$M ginsenoside F2, respectively (Fig. 2B). In addition, phosphorylated smad-2 was 42% and 58% lower than control in the groups treated with 0.1 $\mu$M and 1 $\mu$M ginsenoside F2, respectively (Fig. 2C). Expression of phosphorylated smad-3 was also 56% and 72% lower than control in the respective groups (Fig. 2D). The observed effects of ginsenoside F2 were substantial even compared to those of finasteride, and expression levels of the factors were similar to those seen in cells not treated with DHT.

The expression of Bcl-2 in the ginsenoside F2-treated group was higher than in the controls and the expression of Bax was lower than in the controls. The Bcl-2/Bax ratio was 390% higher in the groups treated with 0.1 $\mu$M and 1 $\mu$M ginsenoside F2, respectively (Fig. 2E).

Expression of caspase-3, a player in the apoptotic cascade, was 57% and 68% lower than controls in the groups treated with 0.1 $\mu$M and 1 $\mu$M ginsenoside F2, respectively, which rep-
Fig. 2. Western Blots Demonstrating the Effect of Ginsenoside F2 (F2) and Finasteride

Effect of F2 and finasteride on DHT-induced expression of sp1 (A), TGF-β2 (B), smad-2 (C), smad-3 (D), Bcl-2/Bax (E), caspase-3 (F), SCAP (G), SREBP (H), and caspase-12 (I) in HHDPCs (Values are mean±S.D.; *p<0.05; **p<0.01; ***p<0.001, treatment compared to DHT; *p<0.05; **p<0.01; ***p<0.001, treatment DHT+finasteride compared to treatment DHT+F2, n=3).
Fig. 3. The Effects of Ginsenoside F2 (F2) and Finasteride on Hair Growth in a DHT-Induced Androgenetic Alopecia Model in C57BL/6 Mice

Analysis of morphological change in a DHT-induced androgenetic alopecia model in C57BL/6 mice treated with DHT only, finasteride or F2 for 35 d. Close-up pictures of the C57BL/6 mice dorsal surfaces (A). Percentage of area showing hair growth measured using the Image J program (B). The back skin of C57BL/6 mice was observed using hair loss diagnosis tools to survey hair density (C) and thickness (D) (Values are mean±S.D.; n=10; *p<0.05; ***p<0.001, treatment compared to DHT; *p<0.05; ***p<0.001, treatment DHT+finasteride compared to treatment DHT+F2.)
represented significant decreases even compared to those seen with finasteride (Fig. 2F).

These results support the hypothesis that ginsenoside F2 prevents DHT-induced hair cell apoptosis, as seen in AGA, which occurs through the mechanism outlined.

Expression of SCAP was 64% lower than in the DHT-treated controls in the group treated with 0.1 µM ginsenoside F2 and 76% lower in the group treated with 1 µM (Fig. 2G). The expression of SREBP was 74% and 91% lower in the groups treated with 0.1 µM and 1 µM ginsenoside F2, respectively, than in the group treated only with DHT (Fig. 2H). Caspase-12 expression was 61% and 82% lower than controls in the groups treated with DHT followed by 0.1 µM and 1 µM ginsenoside F2, respectively (Fig. 2I). Expression of these factors in the ginsenoside F2 treated group after treated was significantly lower than that in the finasteride treated group.

Since caspase-12 is connected to caspase-3, the effects of ginsenoside F2 in preventing DHT-induced hair cell apoptosis as seen in AGA may be in part via a mechanism that interacts with the cholesterol pathway.

**Ginsenoside F2 Reduces DHT-Induced Catagen in C57BL/6 Mice**

**Analysis of Morphological Changes:** The effect of ginsenoside F2 on hair growth in a DHT-induced C57BL/6 mouse model of AGA was assessed. We observed that the group given ginsenoside F2 after depilation and injection of DHT converted their skin color from pink to gray on the 15th day, started to grow hair on the 20th day and were covered with hair by the 28th day. This hair growth timetable was significantly better than that seen in the depilated, DHT-treated mice which received no ginsenoside F2 or finasteride. The group that received finasteride entered anagen 5 d later than the ginsenoside F2-treated group (Fig. 3A).

We also analyzed the extent of hair growth by measuring the percentage of the depilated region that was covered with hair at various time points. The DHT-injected group had 3% hair coverage on the 15th day after depilation, 9% coverage by the 20th day and still showed 9% coverage by the 28th day. In contrast, mice treated with finasteride had 17% hair coverage in the denuded region by the 20th day and 27% by the 28th day after depilation. The group treated with 0.5 mg/kg ginsenoside F2 had 20% coverage by the 15th day, 37% by the 20th day and 54% by the 28th day. The group treated with 2.5 mg/kg ginsenoside F2 had 27% coverage by the 15th day, 51% by the 20th day and 71% on the 28th day (Fig. 3B).

Carefully measurement of hair density revealed increases compared to the DHT-only group of 107% in the group treated with 0.5 mg/kg ginsenoside F2 and 286% in the group treated with 2.5 mg/kg ginsenoside F2. This increase relative to the negative control was only 49% in the finasteride-treated group (Fig. 3C). Hair thickness compared to the DHT-treated negative control group was 33% greater in the finasteride-treated group, 35% greater in the group treated with 0.5 mg/kg ginsenoside F2 and 51% greater in the group treated with 2.5 mg/kg ginsenoside F2 (Fig. 3D).

Analysis of Histological Changes: Mice were sacrificed on the 35th day after depilation and skin tissue samples were stained with H&E for histological analysis. The ginsenoside F2-treated group had longer and overall larger follicles than either the DHT-only group or the finasteride-treated group, both of which had catagen-like follicle morphology (Fig. 4).

The ratio of anagen to telogen in the mouse skin tissue (A/T) was also evaluated. The (A/T) ratios in mice treated with 0.5 mg/kg and 2.5 mg/kg ginsenoside F2 were 2.09±1.17 and 2.25±0.97, respectively, while (A/T) ratios in the DHT-only and finasteride-treated groups were 0.11±0.06 and 0.49±0.17, respectively (Table 1). This implies that ginsenoside F2 can prevent follicles from entering catagen in our DHT-induced AGA mouse model.

**The Inhibitory Effect of Ginsenoside F2 on DHT-Induced Apoptosis in the Skin of C57BL/6 Mice**

Immunohistochemistry was used to assess change in the expression of TGF-β2 and SCAP in the tissue of sacrificed mice. In the ginsenoside F2-treated group, expression of TGF-β2 was observed in the outer root sheath (ORS), as seen in hair follicles in anagen. In contrast, expression was seen in the dermal papilla in the DHT-treated negative control group and in the finasteride-treated group, a pattern which is seen in catagen (Fig. 5).

The expression of SCAP in ginsenoside F2-treated mice was also lower than in the finasteride-treated mice (Fig. 5). These results suggest that ginsenoside F2 hinders expression of apoptotic factors in vivo in the mouse just as it did in HHDPCs in vitro.

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<th>Normal</th>
<th>DHT 30mg/EA</th>
<th>DHT Finasteride 0.5 mg/kg</th>
<th>F2 0.5 mg/kg</th>
<th>F2 2.5 mg/kg</th>
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*Fig. 4. Comparison of Histological Images of Hair Follicle Analysis of histological change in a DHT-induced model of androgenetic alopecia in C57BL/6 mice treated with finasteride or ginsenoside F2 (F2) for 35 d.*
DISCUSSION

This study showed that there was an increase of the proliferation of HHDPCs and HaCaT cells after treatment with either ginsenoside F2 or finasteride. In these experiments, ginsenoside F2 turned out to be more potent in the prevention of hair cell apoptosis induced by DHT than was finasteride. Factors involved with apoptosis including TGF-β, sp1, phosphorylated smad-2 and -3, Bax, cleaved caspase-3 and Bcl-2 are thought to play a major role in the process of hair loss. Among these factors, TGF-β2 is an important for entry to catagen during the hair growth cycle. Western blot analysis of the expression of these factors after treatment with ginsenoside F2 or finasteride provided information about the mechanisms involved. We observed that the induction of TGF-β2, sp1, phosphorylated smad-2, -3, Bax and cleaved caspase-3 by treatment with DHT was quenched by both ginsenoside F2 and finasteride (Fig. 6A). The effects of ginsenoside F2 were consistently more pronounced than those of finasteride.

We attempted to find the new role of these mechanisms in in vitro and in vivo models of DHT-induced AGA. The expression of SCAP was increased by bind of DHT to the AR, ER stress would be induced causing the Golgi apparatus to bind to SREBP. It is known that apoptosis induced by ER

Table 1. Analysis of Hair Growth Pattern (Anagen/Telogen Ratio) in C57BL/6 Mice Treated with Finasteride or Ginsenoside F2 (F2) for 35 d

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<th>DHT 30 mg/EA</th>
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<th>DHT F2 0.5 mg/kg</th>
<th>DHT F2 2.5 mg/kg</th>
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<tr>
<td>Anagen</td>
<td>11.4±1.71</td>
<td>1.3±0.67</td>
<td>6.6±2.67 ***</td>
<td>10.7±2.11 ++</td>
<td>12.5±2.42 +++</td>
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<tr>
<td>Telogen</td>
<td>3.7±0.94</td>
<td>11.8±2.35</td>
<td>13.4±3.17 ++</td>
<td>6.4±2.76 ++</td>
<td>6.2±2.1 ++</td>
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<tr>
<td>A/T ratio</td>
<td>3.34</td>
<td>0.11</td>
<td>0.49</td>
<td>2.09 **</td>
<td>2.25 ***</td>
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Values are mean±S.D.; n=10; *p<0.05; **p<0.01; ***p<0.001, treatment compared to DHT; *p<0.05; **p<0.01; ***p<0.001, treatment DHT+finasteride compared to treatment DHT+F2.

Fig. 5. Comparison of Immunohistological Images of TGF-β2 and SCAP Expression

Analysis of immunohistological change in a DHT-induced model of androgenetic alopecia in C57BL/6 mice treated with oral finasteride or ginsenoside F2 (F2) for 35 d.
stress would result in an increase in the expression of caspase-3 induced by the expression of caspase-12. As shown in Fig. 6B, ginsenoside F2 treatment strongly suppressed DHT-induced expression of SCAP, SREBP and caspase-12, but finasteride treatment slightly decreased it. The decrease in caspase-12 would lead to a decrease of caspase-3, which is our new finding, how ginsenoside F2 finally prevents apoptosis and thus could reduce hair loss.

Because its genetic characteristics are well known, the C57BL/6 mouse is widely used as model for hair growth. Anagen is induced by depilation in these mice. Treatment with DHT causes hair cell apoptosis and entry into catagen and telogen. In Fig. 3, in vivo experiments are described in which the hair coverage, density and thickness after oral administration of ginsenoside F2 were measured. The results of these experiments suggest that ginsenoside F2 may inhibit AGA and hair cell apoptosis. The ratio of follicles in anagen to those in telogen (A/T) in the skin tissue of DHT-treated mice was examined following oral administrations of ginsenoside F2 and finasteride. Ginsenoside F2 was more effective at preventing entry into catagen and hair loss than was finasteride.

Expression of TGF-β2 and SCAP in the skin tissue of C57BL/6 mice was analyzed using IF. Like other groups, we observed that TGF-β2 was expressed in the ORS during anagen, and then moved to the dermal papilla during telogen. Following oral administration of ginsenoside F2, DHT-treated mice, and the expression of TGF-β2 was confirmed to be in the ORS, consistent with a hair follicle in anagen. In contrast, TGF-β2 was expressed in the dermal papilla in mice given finasteride, consistent with hair follicles that are in catagen. Moreover, the expression of SCAP was lower in the group treated with ginsenoside F2 than in the group given finasteride (Fig. 5).

In this study, we looked at the expression of factors involved in apoptosis, AR-pathway-related factors in DHT-treated dermal papilla cells. Hair growth rate, density, thickness and the expression of TGF-β2 and SCAP were also analyzed in the skin tissue of C57BL/6 mice. Taken together, the results of our experiments suggest that ginsenoside F2 influences factors in several pathways, including SCAP, and may be more effective than finasteride for the treatment of DHT-induced AGA.

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