Introduction

The human hair follicle is a complex structure consisting of an outer root sheath, an inner root sheath, the hair shaft, the bulge and the sebaceous gland. The swollen, bottom part of the hair follicle is called the hair bulb. There highly proliferative matrix keratinocytes produce the keratinized hair shaft. The ectodermal matrix encloses a zone of mesenchymal cells, called the dermal papilla. This well-vascularized zone transfers nutrients to the hair bulb and plays an important role in the hair growth cycle. After hair follicles are generated in embryogenesis, the follicles undergo cyclical growth. A hair growth cycle consists of three phases: anagen, catagen and telogen (Fig. 1). Anagen is the growth phase that lasts about three to five years. Afterwards catagen, the involution phase, follows, lasting a couple of weeks. The cycle terminates with the telogen phase, a resting period of up to 4 months. Dermal papilla cells generate signals that regulate the activity of keratinocytes in the follicular matrix. These keratinocytes stop proliferation at the end of the anagen phase and undergo apoptosis in the catagen phase. The onset of a new growth phase and thus the length of the anagen phase are defined by the release of growth factors from dermal papilla cells. Adjacent, quiescent keratinocyte progenitor cells start to proliferate and to generate a new hair follicle. Hair follicles cycle independently, leading to growing, resting and shedding hairs at the same time. The density and total number of scalp hairs do not change. The proportion of telogen hair is nor-

Abstract

Our hair is permanently in a cyclic process which comprises a growth phase (anagen), a transitional phase (catagen) and a resting period (telogen). Healthy hair is characterized by a constant proportion of 85 to 90% anagen hair. Hair loss happens when the proportion of telogen hair increases, leading to a reduced amount of anagen hair. AnaGain™, a new anti-hair loss ingredient based on pea sprouts (INCI name: Pisum Sativum Sprout Extract, Phenoxethanol, Sodium Benzoate, Aqua) was found in a clinical study with 20 volunteers to clearly increase the number of hairs in the anagen phase and to reduce the amount of telogen hair. After three months’ treatment with a gel containing 4% AnaGain™, the anagen/telogen ratio improved from 4 to 7.2. The result is stronger, healthier, denser-looking hair. In order to study how AnaGain™ works, the gene expression in plucked hair follicles from 10 volunteers was analyzed before and after 14 days’ treatment with a hair balm fluid containing 2% AnaGain™. The expression of selected marker genes important in hair physiology was analyzed using the RT-qPCR method on mRNA extracted from pooled hair bulbs. Also dermal papilla cell marker genes were analyzed because plucked hair follicles contained the dermal papilla tissue at the bottom of the hair bulb and because the cyclic process of hair growth is regulated by signals of dermal papilla cells. In most of the volunteers, AnaGain™ was found to increase several times the expression of fibroblast growth factor-7 (FGF7), a dermal papilla signal instructing hair germ cells to proliferate and initiate a new hair cycle. And noggin, a gene that shortens the telogen phase and initiates a new hair growth phase was even more stimulated. To summarize: AnaGain™ prevents and stops the hair loss process and stimulates hair growth by modulating the expression of the genes of FGF7 and noggin.
Normally 10% to 15% (1). Diffuse hair loss is characterized by a uniform reduction of hair density. Telogen effluvium is a diffuse hair loss during stress with premature development of catagen and telogen follicles and premature termination of anagen follicles. A trichogram analysis shows in this case a significant reduction in the anagen:telogen ratio leading to >25% of telogen hair. Triggers for diffuse hair loss include physiological or emotional stress, a hormonal imbalance or nutritional deficiencies.

Materials and Methods

Anti-hair loss study with photo-trichogram analysis
The study was conducted with 17 women and 3 men, aged between 21 and 37. The product, a gel containing 4% AnaGain™, was applied to the scalp twice daily for 12 weeks. The selected subjects showed increased hair loss with ≥20% telogen hair for men and ≥15% telogen hair for women and with a hair density of at least 150 hairs/cm². To perform the phototrichogram, an image was made at the start, immediately after shaving 1 cm² of scalp. Another image was made on day 2. Product application was started on day 3. Another phototrichogram was performed at the end of the study.

Gene expression analysis in plucked hairs
The study was conducted with 10 volunteers, 4 women and 6 men, aged between 46 and 60. AnaGain™ was formulated at 2% into a neutral scalp product. The treatment with the product and the extraction of hair bulbs was performed on a test site at the back of the head. The product was applied twice a day for 2 weeks. Before the first product application and again at the end of the study 20 hairs were plucked from the test site and pooled. The plucked hair bulbs were cut to about 1 cm length and stored at -80 °C. The expression of selected markers was analyzed using RT-qPCR me-

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Method on mRNA extracted from the two hair pools. Analysis of gene expression was performed in duplicates (n=2) using a dedicated PCR array containing 32 target genes (including 2 housekeeping genes) selected for their importance in hair physiology. The PCRs were performed with the LightCycler® system (Roche Molecular System Inc.).

Results and Discussion

Effect of AnaGain™ on Hair Growth

Pea sprouts that were a few days old were used as the raw material to produce the ingredient AnaGain™ (INCI name: Pisum Sativum Sprout Extract, Phenoxethanol, Sodium Benzoate, Aqua), intended for the use as an anti-hair loss ingredient. AnaGain™, formulated at 4% into a gel base, was tested on twenty, mainly female, subjects. The test product was applied on a defined scalp zone, twice a day, over 3 months. Before and after treatment a phototrichogram was performed to analyze hair growth. To perform a phototrichogram, a defined scalp zone is shaved and then photographed. A typical picture is shown in Fig. 2. The number of small dots corresponds to total hair follicles. Two days later, the shaved zone is photographed again in order to verify the amount of hair follicles in the growing phase (anagen hair).

In Fig. 2, hair follicles in the growing phase are marked with a green point. As shown in Fig. 3, treatment of the scalp with AnaGain™ clearly reduced the density of telogen hair (-28.3%) and increased the density of anagen hair (+7.9%). Consequently, the hair growth coefficient which is defined as the ratio of anagen to telogen hair, increased from 4 to 7.2 (Fig. 4). The slightly enhanced hair loss at the beginning of the study (about 20% telogen hair) of the subjects could be normalized by a three month treatment with AnaGain™ (about 12% telogen hair at the end of the study). The volunteers were asked to evaluate the efficacy of the treatment in a questionnaire at the end of the study. 95% of the volunteers noticed a slight to strong reduction in hair loss and a slight to strong improvement of the general hair condition (Fig. 5). 85% of the volunteers noticed renewed hair growth.

Fig. 2 Typical pictures of a phototrichogram analysis

Fig. 3 After three months' treatment, AnaGain™ at 4% clearly reduced the number of telogen hair and increased the number of anagen hair

Fig. 4 Effect of AnaGain™ at 4% on the hair growth coefficient (ratio of anagen to telogen hair) after application for three months
Effect of AnaGain™ on Gene Expression Analyzed in Plucked Hairs

In order to study the mechanism of the action of AnaGain™, a clinical study was designed which allowed the effect of AnaGain™ on gene expression in vivo to be analyzed. AnaGain™ formulated at 2% into a neutral scalp product was applied in the morning and evening over two weeks on 10 subjects. A pool of 20 follicles, plucked before and after treatment, was sufficient to extract enough RNA for a quantitative gene expression profile. Thirty genes important for hair physiology were followed. The results showed mainly modulation of the expression of fibroblast growth factor 7 (FGF7) and of noggin (Fig. 6). AnaGain™ was found to strongly activate both genes. The expression of FGF7 was increased by 56% and of noggin by 85% on average after two weeks' treatment. FGF7 was stimulated in 8 and noggin in 7 out of the 10 volunteers. The expression products of the genes FGF7 and noggin, the FGF7 and noggin proteins, are well known signaling compounds, important for the induction of a new hair growth phase. Both proteins are mainly synthesized in dermal papilla cells. The plucked hair follicles used for the gene expression analysis contained the entire hair bulb enclosing the dermal papilla. So, the extracted RNA came not only from the matrix keratinocytes but also from the neighboring mesenchymal papilla cells. Fig. 7 shows the roles of noggin and FGF7 in the hair follicle cy-

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secondary metabolites are plant compounds that are not required for the primary metabolic processes like growth and reproduction. The role of secondary metabolites in plants is to protect them from disease, damage, pathogens, drought, salinity, extreme ultraviolet and pollutants. Many of these phytochemicals are known to exert beneficial effects on human health or to play an active role in the amelioration of disease. Isoflavones for example are known to act as phytoestrogens or to exert antioxidant activity. Sprouts are the young shoots that develop from germinating seeds. Because the shoots of a plant are especially vulnerable, secondary metabolites are very abundant at this stage. Sprouts that are a few days old have the highest concentration of healthy nutrients per calorie of any food. The high concentration of isoflavones in the pea sprouts might be responsible for the positive effect on hair growth.

Fig. 6 Effect of AnaGain™ at 2% in a neutral scalp product on expression of fibroblast growth factor 7 and noggin in volunteers A to J after two weeks application

Fig. 7 The role of the fibroblast growth factor 7 and noggin proteins as direct and indirect terminators of the telogen phase

**References**


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