A Special Report on the Hair-Growing Activity of Procyanidin B-2

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Procyanidin B-2 and the Hair-Growing Activity of Proanthocyanidins

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The fear of suffering hair loss is a cause of unhappiness in many individuals. Consequently there is considerable demand for developing a safe and effective hair-growth stimulant. In this paper we report experiments on mice and humans showing the hair-growth stimulating effects of the natural compound procyanidin B-2 from purified apple juice as treatment for male pattern baldness.

Male Pattern Baldness

Human hair follicles are known to undergo hair cycles independently: several years in the anagen phase, followed by several weeks in the catagen phase, and followed by several months in the telogen phase. It is known that the hair follicles of mammals have a species-specific hair growth cycle with the same three phases.

Morphologically, hair follicles change dramatically during the hair cycle progression. The bulb of an anagen-stage hair follicle reaches the subcutaneous tissue; however, the bulb of a telogen-stage hair follicle is located in the dermis layer near the epidermis. To enable reversion from the telogen phase to the anagen phase of the hair cycle, downgrowth of the telogen hair follicles is essential.

The initiation of downgrowth of hair follicles in the telogen phase is thought to be triggered by the activation, by unknown mechanisms, of certain parts of the hair follicles, including the outer root sheath, hair matrix and dermal papilla.

Role of the Outer Root Sheath and the Hair Bulb

Hair is composed of epithelial cells, such as inner root sheath cells, outer root sheath cells and hair matrix cells, and mesenchymal cells such as dermal papilla cells.

Interactions between epithelial cells and mesenchymal cells are considered to be important in the progress of the hair cycle through the anagen, catagen, and telogen phases. These mesenchymal-epithelial interactions are assumed to stimulate germinative cells to proliferate, induce the anagen phase in the hair cycle followed by activation of stem cells, and consequently promote downgrowth of the hair follicles.

Stem cells were advocated by Cotsarelis et al.[16] to be present in the bulge area of the infundibular region of the outer root sheath. The outer root sheath thus appears to play a major role in mature hair follicle formation. For hair fiber elongation following induction of the anagen phase in the hair cycle, activation of specific areas of the hair follicle, such as the bulge area in the outer root sheath and hair matrix in the hair bulb, is essential.

Male pattern baldness is defined as a hair disease characterized by the miniaturization of terminal hairs[1] and the vellus transformation of the hair.[2] It is speculated that male pattern baldness is caused chiefly by changes in hormonal factors such as androgen metabolism and its receptor; and other internal or external factors such as unbalanced diet, exposure to sunlight and mental stress are also known to worsen the condition of the disease.

Proanthocyanidins

History: Proanthocyanidins are a family of condensed tannins known to be widespread in plants, where they are thought to act as a defense against damage by ultraviolet radiation and also help resist attack by insects or microorganisms.[3]

Proanthocyanidins have been used as medications aimed at protecting the capillary vessels,[4] as cosmetics to protect the skin,[5] and as antioxidants in foods and beverages; and epidemiological investigations[6] have recently identified them as active compounds in red wine, thought to be effective in reducing the incidence of heart disease. However, except for our previous reports,[7,8] there are no reports on the hair-growing activity of proanthocyanidins.

Structure: We assembled a collection of 136 pharmaceutically usable plants and edible plants, and divided each plant into several sections such as leaves, roots, seeds and fruit. We then applied solvent extraction to the samples: first by chloroform, then by methanol, and finally by hot water. In all, we prepared more than 1,000 plant extracts.

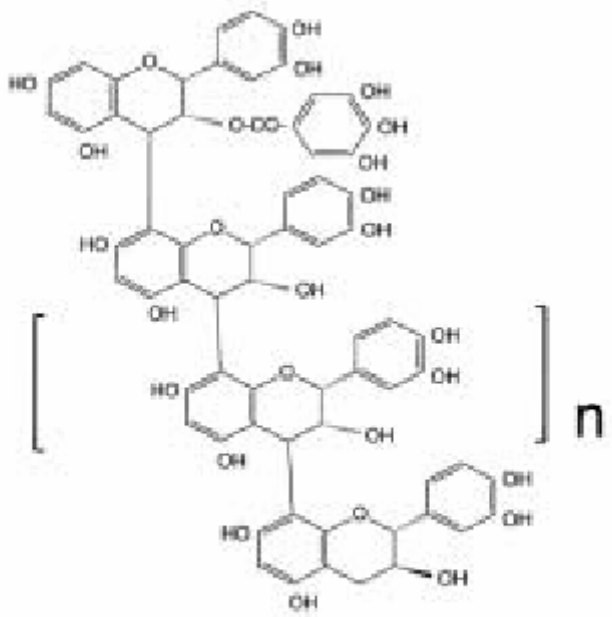

Figure 1. Prospective structure of active fraction of proanthocyanidins purified from grape seeds

We examined those extracts for their growth-promoting activity on hair epithelial cells, and found about 150% growth-promoting activity relative to controls in a methanol extract from grape seeds. We subjected the grape seed (Chardonnay variety) extract (using 70% (v/v) methanol) to HP-20 column chromatography. The 60% (v/v) methanol-eluted active fraction was then subjected to reverse phase ODS preparative HPLC, and we found activity in the 30% (v/v) methanol-eluted fraction. Analyses combining tannase decomposition[9] and toluene-α-thiol decomposition[10] revealed the active components to be proanthocyanidins (Figure 1) whose components were catechin and epicatechin with the characteristics shown in Table 1.

Table 1. Characteristics of the active fraction of proantho-cyanidins purified from grape seeds

		Terminal unit	Extension units
Catechin		1	2.3
Epicatechin		2.8	8.1
Catechin gallate		-	-
Epicatechin gallate		1.3	3.4
Catechin : epicatechin	1 : 4.7		
Degree of polymerization	3.7		
Extent of galloylation	25%		

Hair-growth stimulation in vitro: We demonstrated the hair-growth-promoting activities of a mixture of proanthocyanidins purified from grape seeds at various concentrations in a cell culture system using murine hair epithelial cells. These cells were isolated from C3H/HeNCrj mice[a] dorsal skin and cultured in MCDB 153 medium[11] according to the method reported by Tanigaki et al.[12] with suitable modifications described in another report.[8] Dermal fibroblasts were obtained from the EDTA-trypsin-treated dermis of C3H/HeNCrj mice and cultured in a DMEM medium. For comparison purposes, we ran the same tests on (–)-epicatechin and minoxidil. Epicatechin is a monomeric unit of proanthocyanidins. We examined the growth-promoting activity of (–)-epicatechin to confirm whether hair-growing activity is associated with oligomeric structure of proanthocyanidins.

At optimal concentration, proanthocyanidins purified from grape seeds promote the proliferation of murine hair epithelial cells at about 230% relative to controls. Minoxidil was less effective in this cell culture system, with a proliferative activity of about 160% at optimal concentration (Figure 2).

Procyanidins

Following our experiments with proanthocyanidins, we isolated several procyanidins from apples and barley husks as single compounds in purified form and examined their hair-growing activity. Procyanidin B-2 [epicatechin-(4 βØ8)-epicatechin] and procyanidin C-1 [epicatechin-(4βØ8)-epicatechin-(4βØ8)-epicatechin] (Figure 3) were obtained from apple juice according to the method described elsewhere.[8] Our results identified procyanidin B-2 purified from apples to be a powerful stimulant that promotes hair epithelial cell growth more than 300% relative to controls.

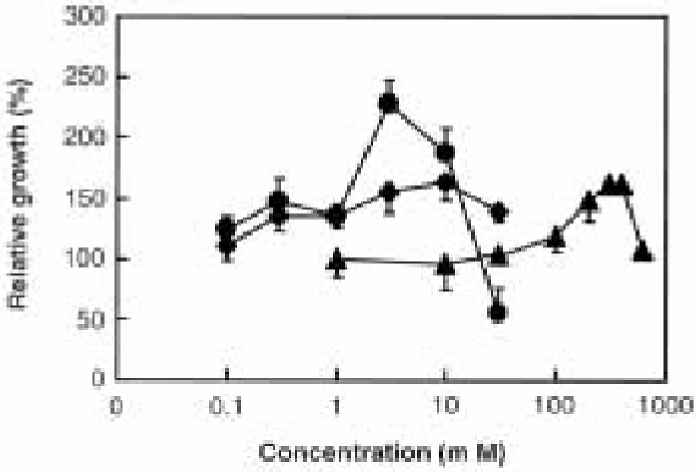

Figure 2. Growth-promoting activity of grape seed proanthocyanidins on C3H mouse hair epithelial cells. (♦), (-)-epicatechin; (•), proanthocyanidins purified from grape seeds; (▲), minoxidil

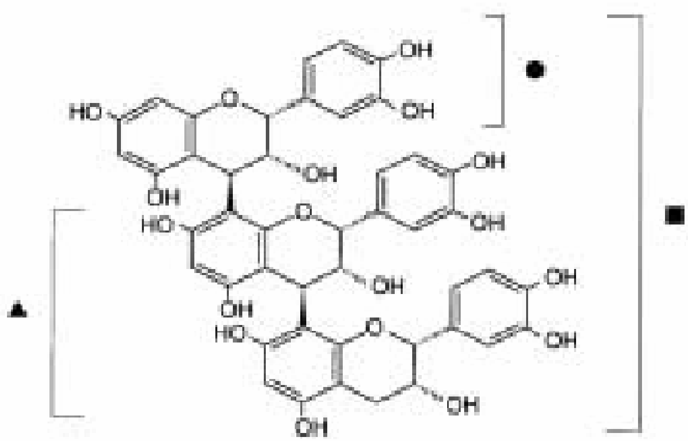

Figure 3. The structure of procyanidin monomer and oligomers. (•), (-)-epicatechin; (▲), procyanidin B-2; (■), procyanidin C-1

Hair-growth stimulation in vitro: We demonstrated the hair-growth-promoting activities of procyanidin B-2 and procyanidin C-1 purified from apples at various concentrations in a cell culture system using murine hair epithelial cells. These cells were isolated and cultured as described above. For comparison purposes, we ran the same test on (–)-epicatechin.

Of the proanthocyanidin species we isolated and whose activity we examined, procyanidin B-2 purified from apples shows the highest activity of more than 300% relative to controls at a concentration of 30 μM. The peak activity of procyanidin C-1 was lower than that of procyanidin B-2; however, its activity at lower concentrations exceeds that of procyanidin B-2. On the other hand, (–)-epicatechin, a flavan-3-ol unit comprising procyanidins, exhibits minimal activity (Figure 4). The growth of dermal fibroblasts from mice and dermal papilla cells from rats (data not shown in either case) was not affected by either procyanidin B-2 or procyanidin C-1 in the concentration range examined (0.01-100 μM).

Hair-growth stimulation in vivo: We demonstrated the hair-growth-promoting activities of procyanidin B-2 and procyanidin C-1 in an in vivo assay that measured induction of the anagen phase in C3H/HeSlc miceb with reference to the method of Hattori and Ogawa.[13] We prepared three topical formulations: two of which contain procyanidins of different degrees of polymerization (procyanidin B-2 or procyanidin C-1) and one of which contains minoxidil. Vehicle without procyanidins or minoxidil was used as the control.

The group to which 1% procyanidin B-2 had been applied showed an extensive growth area of 69.6% ± 21.8% (average ± SD); the group to which 1% procyanidin C-1 had been applied showed an extensive growth area of 78.3% ± 7.6% (average ± SD); the group to which 1% minoxidil had been applied showed an extensive growth area of 81.2% ± 10.5% (average ± SD). On the other hand, the control group to which vehicle was applied showed little hair growth: about 40% (41.7% ± 16.3%, average ± SD) of the shaven area was covered with hair (Figure 5). These results demonstrate that procyanidin oligomers, such as procyanidin B-2 or pro-cyanidin C-1, possess marked hair-growing activity by inducing the anagen phase in vivo.

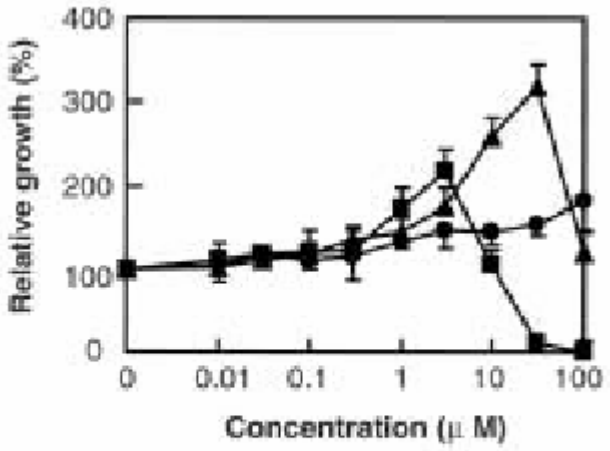

Figure 4. Growth-promoting activity on C3H mouse hair epithelial cells by procyanidins of different degree of polymerization. (•), (-)-epicatechin; (▲), procyanidin B-2; (■), procyanidin C-1

Toxicological studies: To evaluate the safety of topical procyanidin B-2 as a hair growing agent, we examined the mutagenicity, acute subcutaneous injection, primary irritation, skin sensitization, and eye irritation of this compound according to standard methods. No toxicological problems were revealed (Table 2).

Table 2. Toxicological studies on procyanidin B-2

Test item	Organism	Test substance	Results
Mutagenicity			
Reverse mutation	*S. typhimurium, E. coli*	PB2*	non-mutagenic
Chromosomal aberration	CHL cells	PB2	no structural aberration
Micronucleus	mouse	PB2	negative
Primary irritation	rabbit	Preparation	no primary irritation
Acute subcutaneous injection	rat	PB2	LD_{50} > 2000 mg/kg
Skin sensitization	guinea pig	PB2	extremely low
Primary ocular irritation	rabbit	Preparation	slight irritation**

*PB2 = Procyanidin B-2
** = same as placebo

Clinical Trial on Humans

We conducted a 6-month clinical trial focusing on topical application of procyanidin B-2 of high purity to test whether it possesses a curative effect on male pattern baldness.

Materials and methods: We isolated procyanidin B-2 to a purity exceeding 94% (w/w) from apple juice and used it in our clinical test. The placebo-controlled clinical trial was performed in a total of 30 subjects (volunteer employees at the Kyowa Hakko Kogyo Co.'s Tsuchiura Plant in Ibaraki, Japan) showing male pattern baldness on the head. Nineteen men (one employee was transferred to a different location and dropped out) in the procyanidin B-2 group and 10 men in the placebo control group were subjected to analyses after confirming that there were no significant differences between the two groups as to background factors such as age or type and degree of baldness (Table 3).

The tests were performed in double-blind fashion. A topical formulation (Formula 2) containing 1% (w/w) procyanidin B-2 (about 1.8 ml per dose) was applied to the subjects' affected scalp area twice a day, giving a daily dose of 30 mg of procyanidin B-2. Vehicle without procyanidin B-2 was used as the placebo control.

During the 6 months of twice-daily application of the agent, the hair-growing effects were evaluated according to the following parameters:

Table 3. Background factors

Group	Number of subjects	Mean age	Patterns of baldness[a]		Degrees of baldness[b]		
			Type II	Type IV	Slight	Moderate	Severe
Placebo	10	48	6	4	2	6	2
PB2*	19	45	11	8	4	11	4

[a]According to the Ogata scale[23,24]
[b]Placed in three ranks: slight, moderate, and severe
*PB2 = procyanidin B-2

- The macrophotographically recorded change in the number of hairs in the designated scalp area;
- The changes in the diameter of hairs clipped from the designated scalp area;
- The changes in the photographically recorded global view of the subjects' head.
- At the same time, its effects on scalp condition were checked to identify side effects, if any.

Changes in hair density: In the procyanidin B-2 group, the increase in the number of total hairs in the designated scalp area (0.5 cm square = 0.25 cm^2) after the 6-month trial was 6.68 ± 5.53 (mean ± SD)/0.25 cm^2, whereas in the placebo control group, the increase in number of total hairs was 0.08 ± 4.55 (mean ± SD)/0.25 cm^2 (Table 4).

Table 4. Effects on hair growth

Group		The number of hairs Increase of hairs in 0.25 cm^2			Hair diameter Increased ratio of hairs (%)	
		Total hairs	Non-vellus hairs (>40 μm)	Terminal hairs (>60 μm)	Non-vellus hairs (>40 μm)	Terminal hairs (>60 μm)
Placebo	Mean	0.08	-1.63	-0.82	-4.07	-3.41
	SD	4.55	7.43	3.40	18.07	11.33
PB2*	Mean	6.68	5.79	1.99	6.52	3.06
	SD	5.53	4.85	2.58	11.89	8.13
	p**	<0.005	<0.005	<0.02	<0.1	<0.1

*PB2 = procyanidin B-2
** two-sample *t*-test

It is calculated that the increased number of total hairs in the designated scalp area of the procyanidin B-2 group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects ($p < 0.005$, two-sample t-test). The number of total hairs in the designated scalp area (0.5 cm square = 0.25 cm^2) after 6 months of procyanidin B-2 treatment significantly increased over the baseline figure for each subject ($p < 0.001$, paired t-test); on the other hand, no significant difference was observed in the placebo controls (paired t-test) (Figure 6).

Changes in hair diameter: In the procyanidin B-2 group, the increase in the number of non-vellus hairs (hairs > 40 μm in diameter) in the designated scalp area (0.5 cm square = 0.25 cm^2) after the 6-month trial was 5.79 ± 4.85 (mean ± SD)/0.25 cm^2; whereas in the placebo control group, the increase in number of non-vellus hairs was -1.63 ± 7.43 (mean ± SD)/0.25 cm^2 (Table 4). It is calculated that the increased

number of non-vellus hairs in the designated scalp area of the procyanidin B-2 group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects ($p < 0.005$, two-sample t-test).

In the procyanidin B-2 group, the increase in number of terminal hairs (hairs > 60 μm in diameter) in the designated scalp area (0.5 cm square = 0.25 cm^2) after the 6-month trial was 1.99 ± 2.58 (mean ± SD)/0.25 cm^2; whereas in the placebo control group, the increase in number of terminal hairs was -0.82 ± 3.40 (mean ± SD)/0.25 cm^2 (Table 4). It is calculated that the increased number of terminal hairs in the designated scalp area of the procyanidin B-2 group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects ($p < 0.02$, two-sample t-test).

The increased ratio of non-vellus hairs measuring more than 40 μm in diameter after 6 months of procyanidin B-2 treatment was higher than that of the placebo controls ($p < 0.1$, two-sample t-test) (Table 4). The increased ratio of terminal hairs measuring more than 60 μm in diameter after 6 months of procyanidin B-2 treatment was higher than that of the placebo controls ($p < 0.1$, two-sample t-test) (Table 4).

Global photographic assessment: After the 6-month trial, the scores of global photographic assessment of procyanidin B-2 treatment group were significantly higher than those of the placebo controls ($p < 0.05$, Mann-Whitney U-test) (Figure 7).

Side effects: Dermatological diagnoses revealed no adverse side effects. No patients complained of itchiness, pain, dryness or scaling of the scalp.

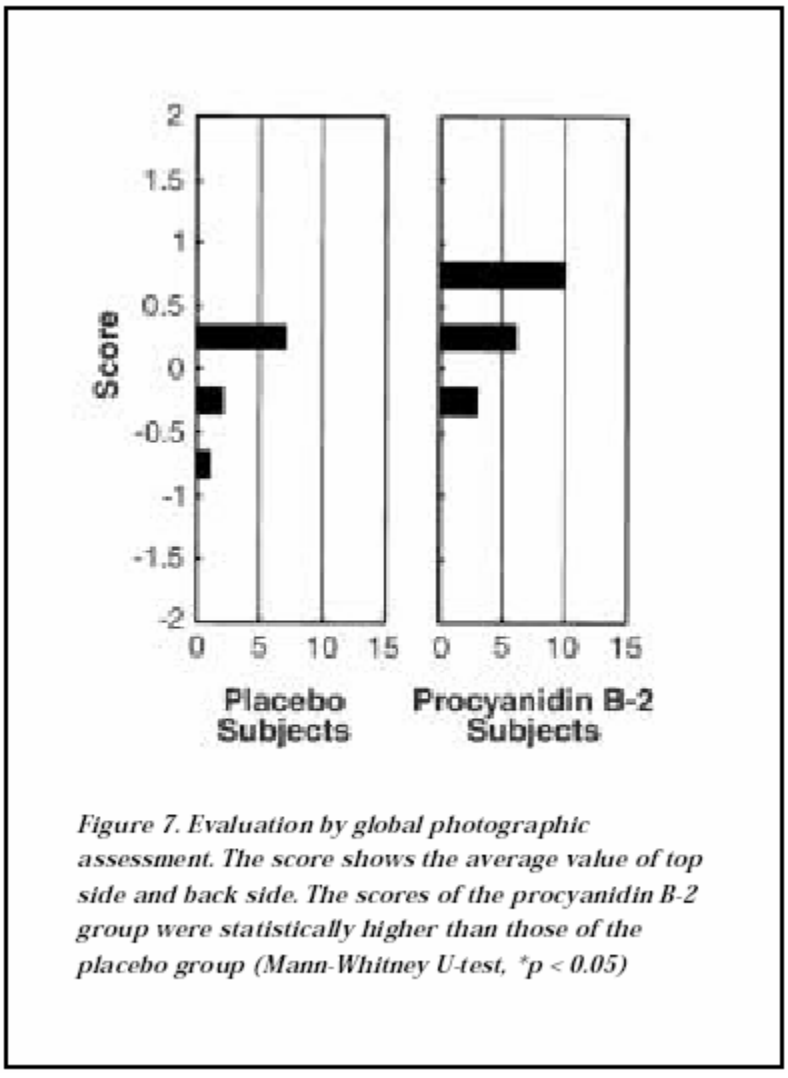

*Figure 7. Evaluation by global photographic assessment. The score shows the average value of top side and back side. The scores of the procyanidin B-2 group were statistically higher than those of the placebo group (Mann-Whitney U-test, *p < 0.05)*

Discussion

Proanthocyanidins:
Proanthocyanidins are polyphenol compounds, widely present in the plant kingdom. The reason for their presence in plants is thought to be their ability to protect the plant cells from oxidation by radicals produced by UV radiation.[3] Proanthocyanidins have recently attracted much attention as healthful compounds in red wine which are thought to reduce the incidence of heart disease; and are considered to be one of the factors which explain the epidemiological phenomenon known as the "French paradox."[14]

Although proanthocyanidins are known to possess a variety of physiological and pharmacological effects,[15] there have been no reports on the hair-growing activity of proanthocyanidins except for our previous reports.[7,8] In addition, proanthocyanidins in commercial use comprise a mixture of many proanthocyanidin molecules and other compounds.

After an extensive search for natural products possessing hair-growing activity, we found proanthocyanidins to be active compounds. We assembled a collection of 132 pharmaceutically useable plants and edible plants, and prepared more than 1,000 plant extracts.

After proceeding with purification steps and a long series of growth-promoting assays on hair epithelial cells, we found procyanidin B-2, an (–)-epicatechin dimer, to be a powerful stimulator of hair epithelial cell growth. We suggest that it specifically promotes hair epithelial cell growth (see sidebar), activates specific regions of the hair follicle into the growing phase, and causes the follicular hair cycle to revert to the anagen phase from the telogen phase. Thus, it is possible to assume that the procyanidin oligomers, such as procyanidin B-2, which directly affect hair epithelial cell growth and possess anagen-inducing activity in vivo, will be effective in curing male pattern baldness.

To test this concept, we obtained procyanidin B-2 in highly purified form from apple juice and investigated the effects on hair growth in a 6-month clinical trial of topical application of 1% (w/w) procyanidin B-2.

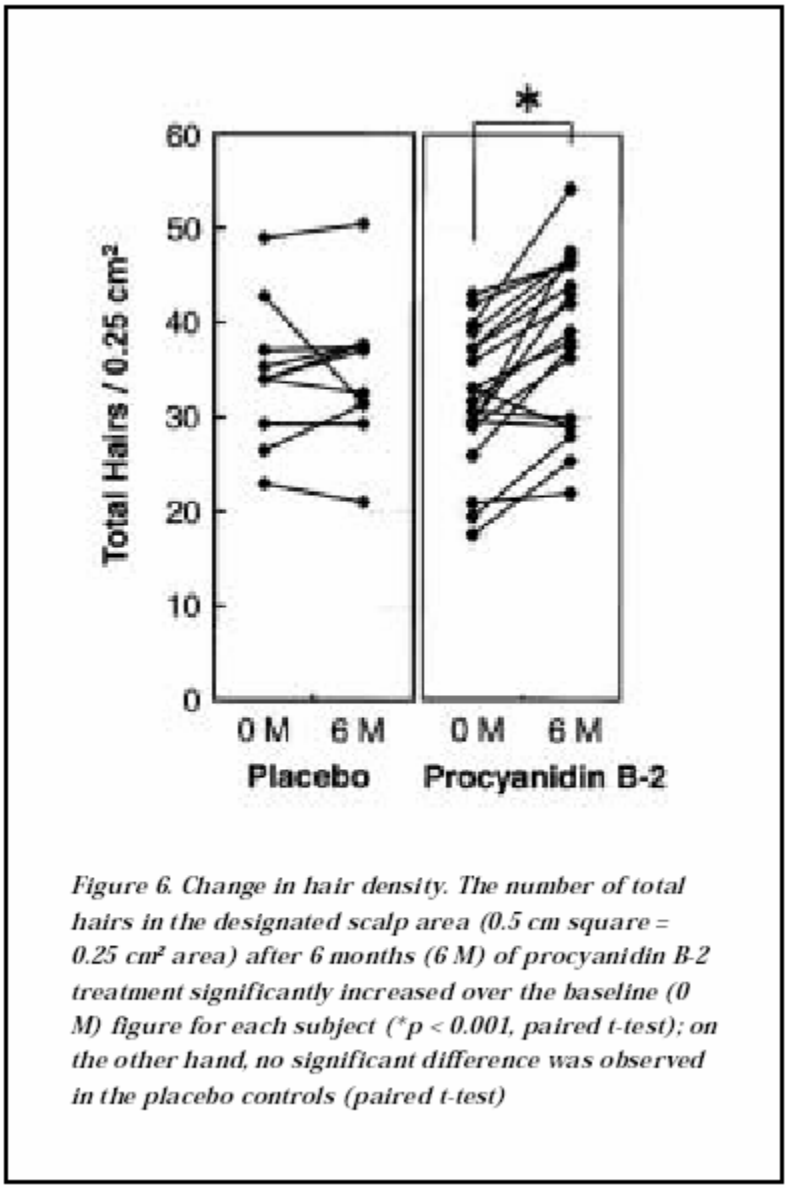

*Figure 6. Change in hair density. The number of total hairs in the designated scalp area (0.5 cm square = 0.25 cm² area) after 6 months (6 M) of procyanidin B-2 treatment significantly increased over the baseline (0 M) figure for each subject (*p < 0.001, paired t-test); on the other hand, no significant difference was observed in the placebo controls (paired t-test)*

Efficacy of procyanidin B-2 on hair growth: Male pattern baldness is defined as a hair disease characterized by the miniaturization of terminal hairs1 and the vellus transformation of the hair.2 In a clinical trial, we examined the number of total, non-vellus, and terminal hairs in the designated scalp area by macrophotography and measurement of the diameter of clipped hairs.

In spite of the small number of subjects and the short period over which the trial was carried out, we observed a clear trend and significant results which were revealed by statistical analyses, indicating increased hair density and hair diameter in the procyanidin group results (Figure 6, Table 4).

It is thought that the application of procyanidin B-2 causes an increase in the anagen ratio, leading to increased hair density and diameter. The same effects have been reported with minoxidil therapy for androgenetic alopecia.[17-19] In 2% minoxidil treatment, an increase of 250 total hairs/5.1 cm^2 (calculated as 12.3 total hairs/0.25 cm^2) after 12-month therapy was reported.[19] We observed an increase of 6.7 total hairs/0.25 cm^2 after 6 months of procyanidin therapy (Table 4). The level of efficacy of 1% procyanidin B-2 is concluded to compare favorably with that of minoxidil therapy.

Assumed mechanism of action of procyanidin B-2: The main mechanism of action of procyanidin B-2 is implied by its intensive growth-promoting action on hair epithelial cells,[8] presumably inducing the anagen

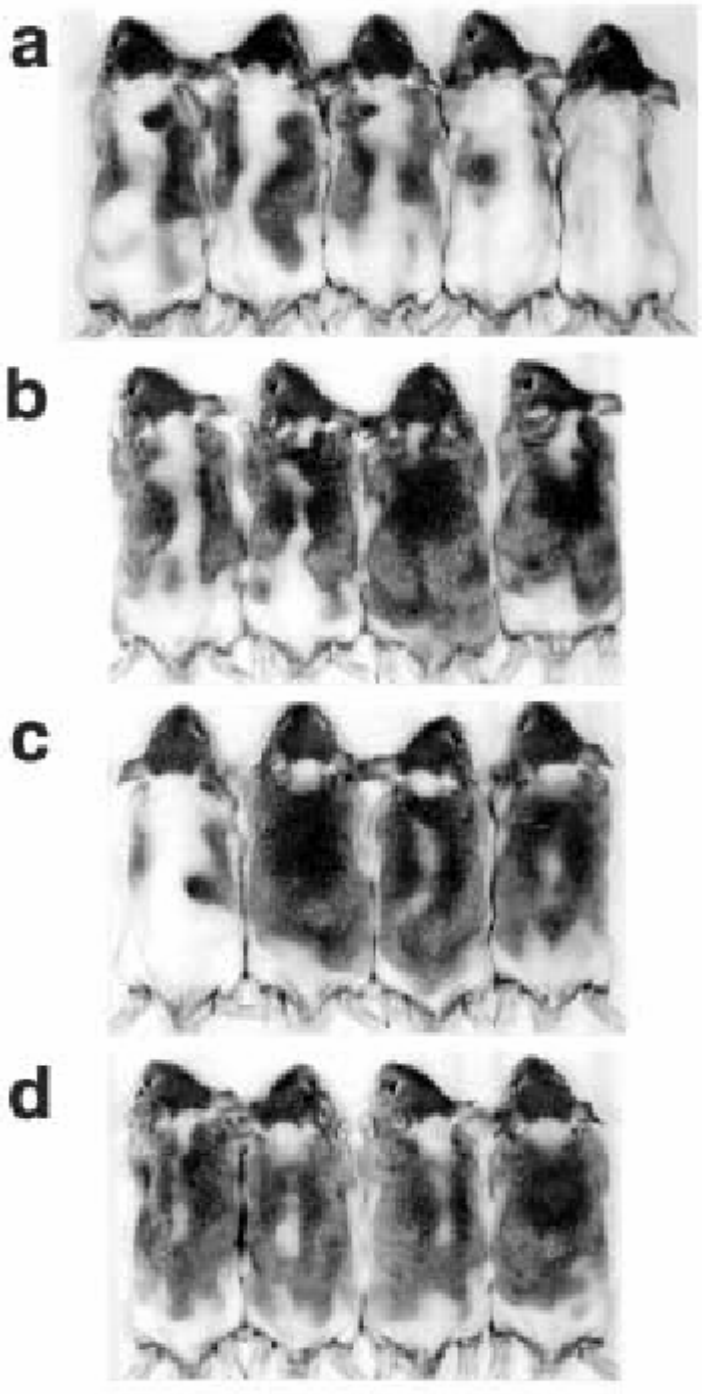

Figure 5. Hair-growing activity evaluated using the C3H mouse in vivo model. (a), vehicle; (b), 1% minoxidil; (c), 1% procyanidin B-2; (d), 1% procyanidin C-1

phase of the hair cycle by the activation of the hair germ, activating the outer root sheath that includes stem cells, and consequently promoting downgrowth of the hair follicles. In addition, the intensive anti-oxidative activity of procyanidin B-2 may be a significant contributor to its effects.

The relation between male pattern baldness and inflammation has been pointed out by several researchers.[20,21] Procyanidins are known to show the effect of reducing inflammation due to their anti-oxidative properties[15] and their protease inhibiting action.[22] Therefore, it is supposed that the suppression of inflammation mediated by procyanidin B-2 returns the scalp to a healthy condition, consequently leading to a cure for baldness.

The hair-growing activity of procyanidin B-2 may depend on more than one of the numerous physiological functions of this compound.

Conclusion

In murine models, we have demonstrated that procyanidin oligomers intensively promote hair epithelial cell growth. We believe they stimulate hair growth by activating telogen hair follicles and causing the follicular hair cycle to revert to the anagen phase from the telogen phase.

In a clinical trial over 6 months, we showed that topical treatment with procyanidin B-2 significantly increased the number of hairs (total, non-vellus, terminal) compared to treatment with a control. The active treatment also increased the ratio of non-vellus hairs (hairs > 40 μm in diameter) and terminal hairs (hairs > 60 μm in diameter), compared to treatment with a control.

Our tests have confirmed that topical procyanidin B-2 is safe and effective in curing male pattern baldness.

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