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The First Clinical Trial of Topical Application of Procyanidin B-2 to Investigate its Potential as a Hair Growing Agent

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Procyanidin B-2 is a compound we have identified in apple which acts as a growth-promoting factor on murine hair epithelial cells. This report describes our investigation of the hair-growing effects of 1% procyanidin B-2 tonic after sequential use for 4 months. A double-blind clinical trial was performed, involving a total of 29 subjects (procyanidin B-2, 19 men; placebo, 10 men). No adverse side effects were observed in either group. In the procyanidin B-2 group, 78.9% showed an increased mean value of hair diameter, whereas only 30.0% in the placebo group showed any increase ($p < 0.02$, Fisher's exact probability test). The increased ratio of hairs measuring more than 40 μm in diameter after 4 months of procyanidin B-2 treatment was significantly higher than that of the placebo controls ($p < 0.05$, two-sample *t*-test). The increase in number of total hairs in the designated scalp area (0.25 cm^2) of procyanidin B-2 subjects after a 4 month trial was significantly greater than that of the placebo controls (procyanidin B-2, 3.67 ± 4.09 (mean $\pm$ SD)/0.25 cm^2; placebo, -2.54 ± 4.00/0.25 cm^2; $p < 0.001$, two-sample *t*-test). Procyanidin B-2 therapy shows potential as a promising cure for male pattern baldness. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: androgenetic alopecia; condensed tannin; external application; *Malus pumila*; proanthocyanidins; scalp.

INTRODUCTION

Proanthocyanidins are a species of polyphenol with many reported pharmacological effects (Haslam, 1996). They have been used as skin-protective cosmetics (Wayne, 1996) and as a treatment for capillary stabilization (Brasseur, 1989; Dartenuc *et al.*, 1980). We have reported that procyanidin oligomers such as procyanidin B-2 (Fig. 1) possess growth-promoting activity in murine hair epithelial cells at a very high rate of 300% relative to controls, and have also demonstrated that procyanidin oligomers stimulate anagen induction in the murine model at almost the same intensity as minoxidil (Takahashi *et al.*, 1998; Takahashi *et al.*, 1999a). We isolated procyanidin B-2 to a purity exceeding 94% (w/w) from apple juice and subjected it to a series of toxicological studies (Takahashi *et al.*, 1999b). Our results confirm the safety of topical application of procyanidin B-2 to human skin. This is the first report to investigate the effects of topical procyanidins on the scalp and hair, and the effects of a highly purified procyanidin dimer on humans. We report here the first clinical trial to focus on the topical application of procyanidin B-2 as a scalp condition improver and its potential for curing male pattern baldness. The aims and objectives of this study were to identify any remedial effects on male pattern baldness and to investigate the desirability of proceeding to large-scale clinical trials of topical procyanidin B-2.

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MATERIALS AND METHODS

Patients. To investigate the effects of topical application of procyanidin B-2 on the scalp and hair, a placebo-controlled clinical trial was performed at the company (Tsuchiura Plant, Kyowa Hakko Kogyo Co., Ibaraki, Japan) on volunteer employees. From 50 applicants, 30 volunteer subjects (30–57 years old, in good health) were

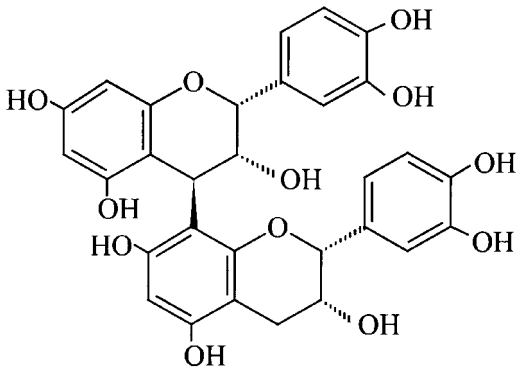

Figure 1. Structure of procyanidin B-2 [epicatechin-(4$\beta \rightarrow$8)-epicatechin].

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chosen by pre-examination under the criteria that they showed male pattern baldness on the scalp, had no dermatological disorders other than male pattern baldness on the scalp, had no other diseases and were not undergoing any medical treatment. The pattern of baldness was classified according to the Ogata scale (Ogata, 1953; Takashima *et al.*, 1981) specific to Japanese males. They were allocated by a dermatologist, one of the authors, to one of three groups (slight, moderate and severe) according to their degree of baldness; and then randomly divided into a 1:2 ratio (placebo:treatment) within each group by random sampling (Snedecor and Cochran, 1967) using computer software (Excel, Microsoft, USA). The test was then started 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.

Study schedule. One group (20 men) was treated with 1% procyanidin B-2 agent, and the other group (10 men) was treated with a placebo control. For 4 months, 1.8 mL of the test agent was applied to the subjects' affected area of the head twice a day, resulting in a daily dose of 30 mg of procyanidin B-2. No use of other hair care products except shampoos and rinses was permitted during the clinical trial. The test was performed in a double-blind fashion.

Determination of change in hair diameter. Before and after the test, hairs at a predetermined site (a round area 1 cm in diameter) of the subjects were clipped with small straight surgical scissors (Tsuji *et al.*, 1994). The site was selected from the outskirts of the affected area on the vertex of each subject using a plastic template connected by a strut to the frame of a pair of eyeglasses. The diameters of the bases of the collected hairs were measured using a micrograph-equipped microscope (BH-2, Olympus Optical Co., Tokyo, Japan) at a magnification of $\times 300$ (Rushton *et al.*, 1983). The analyses were all performed by investigators in a double-blind fashion. Thus, the ratio of terminal hairs, which are defined as hairs more than 40 μm in diameter (Rushton *et al.*, 1983), was determined.

Determination of change in hair density. The hair-cutting sites were photographed using a camera (OM-4 Ti, Olympus Optical Co., Tokyo, Japan) fitted with a macro lens (Zuiko Auto-macro 20 mm, F2, Olympus Optical Co., Tokyo, Japan). The hairs in the photograph of this specific area (0.5 cm square = 0.25 cm^2 area on natural size) were counted by three independent investigators three times each in a double-blind fashion.

Diagnosis by dermatologist. During the test, all subjects underwent a clinical diagnosis by one of the authors, a dermatologist, focusing on any adverse dermatological reactions such as inflammation, erythema or eczema; any effects on scalp condition such as dandruff or sebum secretion; and for any effects on baldness such as the emergence of terminal hairs or the changes in the ratio of vellus hair versus terminal hair. The test was performed in a double-blind fashion.

Statistical analysis. The differences in changes in mean hair diameter, increased ratio of terminal hairs (>40 μm), and total hair increase between the placebo group and procyanidin B-2 group were analysed using the two-sample *t*-test. For the between-group comparison of the number of subjects in which hair-growing effects (change in mean hair diameter: ≥ 0 μm; increased ratio of terminal hairs (>40 μm): $\geq 0\%$; total hair increase: ≥ 0; diagnosis by a dermatologist: positive; subjects' perception: positive) were observed, Fisher's exact probability test was used. Total hair increase and terminal hair increase after the 4 month trial period compared with the baseline of every subject in each group were analysed using the paired *t*-test. All the differences were considered significant at a level of $p < 0.05$.

Product characteristics. Procyanidin B-2 [epicatechin-(4$\beta \rightarrow 8$)-epicatechin] (213 g; purity, >94% (w/w)) was obtained from 20 kL of apple juice (*Malus pumila* Miller var. *domestica* Schneider, Fuji variety, commercial juice) according to the method described in a previous report (Takahashi *et al.*, 1999a). The product was identified using mass spectrometry, ^{1}H-NMR and ^{13}C-NMR (Thompson *et al.*, 1972; Morimoto *et al.*, 1986).

Preparation of hair tonic for human clinical trial. One percent (w/w) of procyanidin B-2, 70% (w/w) of ethanol, 10% (w/w) of 1,3-butylene glycol, 0.5% (w/w) of *N*-acetylglutamine isostearylester (Kyowa Hakko Kogyo Co., Japan), 0.25% (w/w) of polyoxyethylene (25) glyceryl monopyroglutamate monoisostearate (Nihon Emulsion Co., Japan), 0.1% (w/w) of *dl*-α-tocopherol, 0.05% (w/w) of *d*-biotin, 0.1% (w/w) of ascorbyl palmitate, 0.001% (w/w) of β-carotene, 0.1% (w/w) of sodium citrate, and 17.899% (w/w) of purified water were uniformly mixed to prepare the test sample. Vehicle without procyanidin B-2 was used as the placebo control. The placebo preparation was identical to the treatment preparation in smell, appearance and consistency; ensuring that the subjects would be unable to identify whether their test sample was the placebo or the treatment preparation. In addition, both preparations were put into the same black, opaque bottles.

Compliance. A useful measure for the subjects was the consumption of one 50 mL bottle within 2 weeks. The amount of agent habitually used was checked by the weighing the bottle after 2 weeks of usage. Additionally, the subjects were obliged to keep a diary on the use of the agent and any observations in connection with it.

Ethical approval. Results of toxicological studies on procyanidin B-2 (Takahashi *et al.*, 1999b) indicate that the safety of topical procyanidin B-2 has been thoroughly secured. Additionally, we performed an allergy test (challenge test) on 44 volunteers by means of 24 h closed patches after a 2 week sequential application of 1% (w/w) procyanidin B-2 preparation following a 2 week period of no treatment. The results were all negative. The proposed human volunteer test passed Kyowa Hakko Kogyo Co., Ltd.'s Safety Commission. An Informed Consent contract was agreed between individual subjects and the company, confirming their willingness to participate in the test, their freedom to drop out at any time, confirming the acceptance by the company of responsibility for any accidents caused by procyanidin B-2 application, and safeguarding the confidentiality of individual information.

Table 1. 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 (Ogata, 1953; Takashima *et al.*, 1981).
[b] Placed in three ranks: slight, moderate and severe.
PB2: procyanidin B-2.

Table 2. Effects on hair growth

Group		Hair diameter		Total hairs
		Change of mean value (μm)	Increased ratio of hairs >40 μm (%)	Increase of hairs in 0.25 cm^2
Placebo	Mean	−1.08	−4.32[a]	−2.54[b]
	SD	6.77	18.31	4.00
Procyanidin B-2	Mean	2.68	8.04[a]	3.67[b]
	SD	4.68	13.75	4.09

[a] $p < 0.05$, two-sample *t*-test.
[b] $p < 0.001$, two-sample *t*-test.

RESULTS

Withdrawals

Data were available from 29 of the 30 patients who began the trial. One person in the procyanidin B-2 group was transferred to another location and thus dropped out. At the end of the test, 19 men in the procyanidin B-2 group and 10 men in the placebo group were subjected to analyses. No other persons dropped out for any reason.

Background factors

We confirmed that there were no significant differences between the procyanidin B-2 group and the placebo control group subjects with regard to distribution of background factors such as age or type and degree of baldness (Table 1).

Changes in hair diameter

The increased ratio of hairs measuring more than 40 μm in diameter, which is defined as non-vellus hair (Rushton *et al.*, 1983), after 4 months of procyanidin B-2 treatment was significantly higher than that of the placebo controls ($p < 0.05$, two-sample *t*-test) (Table 2). In nearly 80% of the subjects in the procyanidin B-2 group (78.9%), we observed an increased mean value of hair diameter, whereas 30.0% in the placebo group showed an increased mean value of hair diameter ($p < 0.02$, Fisher's exact probability test) (Table 3).

Changes in hair density

The increase in the number of total hairs in the designated scalp area (0.25 cm^2) of procyanidin B-2 subjects after a 4 month trial was significantly greater than that of the placebo controls (procyanidin B-2, 3.67 ± 4.09 (mean ± SD)/0.25 cm^2; placebo, -2.54 ± 4.00/0.25 cm^2;

Table 3. The number of subjects in which hair-growing effects were observed

Group	Number of subjects	Hair diameter		Total hairs	Hair growth	
		Change of mean value ≥0 μm	Increased ratio of hairs (>40 μm) ≥0%	Increase of hairs in 0.25 cm^2 ≥ 0	Diagnosis [Positive]	Subjects' perceived [Positive]
Placebo	10	3[a] (30%)	5 (50%)	2[b] (20%)	3 (30%)	2[c] (20%)
PB2	19	15[a] (78.9%)	15 (78.9%)	16[b] (84.2%)	8 (42.1%)	12[c] (63.2%)

PB2: procyanidin B-2.
[a] $p < 0.02$, Fisher's exact probability test.
[b] $p < 0.002$, Fisher's exact probability test.
[c] $p < 0.05$, Fisher's exact probability test.

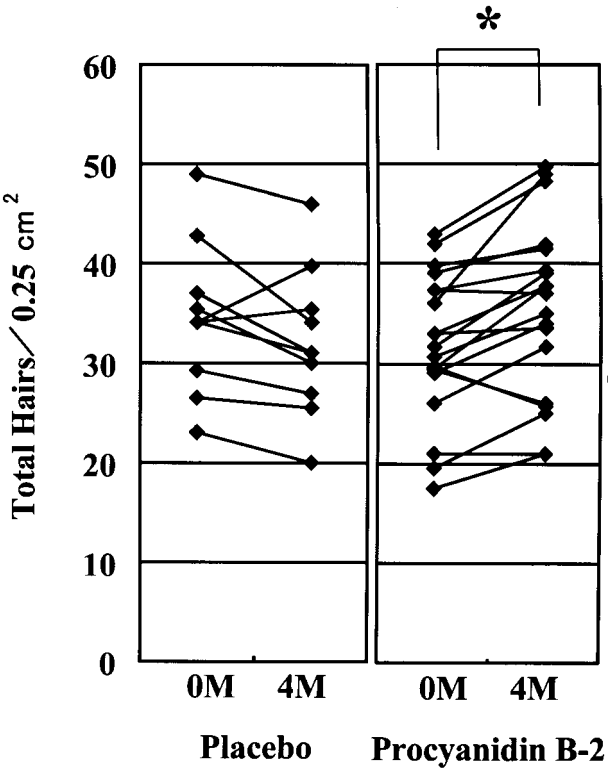

Figure 2. Change in hair density. The number of total hairs in the designated area (0.5 cm square = 0.25 cm^2 area) after 4 months (4M) of procyanidin B-2 treatment significantly increased over the baseline (0M) figure for each subject (*$p < 0.002$, paired *t*-test); on the other hand, no significant difference was observed in the placebo controls (paired *t*-test).

$p < 0.001$, two-sample *t*-test) (Table 2). In more than 80% of the subjects in the procyanidin B-2 group (84.2%), we observed an increased hair density, whereas 20.0% in the placebo group showed an increased hair density ($p < 0.002$, Fisher's exact probability test) (Table 3). The number of total hairs in the designated area (0.5 cm square = 0.25 cm^2 area) after 4 months of procyanidin B-2 treatment significantly increased over the baseline figure for each subject ($p < 0.002$, paired *t*-test); on the other hand, no significant difference was observed in the placebo controls (paired *t*-test) (Fig. 2).

Diagnosis on hair growth

Of the subjects in the procyanidin B-2 group, 42.1% were positively evaluated; on the other hand, 30% of placebo subjects were positively evaluated (Table 3).

Hair growth demonstrated by the subjects

More than 60% (63.2%) of subjects in the procyanidin B-2 group demonstrated hair-growing effects after the 4 month trial; on the other hand, only 20% in the placebo group demonstrated hair-growing effects after sequential use of the agent. The percentage of the procyanidin B-2 group demonstrating positive results was significantly higher than that of the placebo controls ($p < 0.05$, Fisher's exact probability test) (Table 3).

Dermatological diagnoses of scalp condition

Dermatological diagnosis revealed no adverse side effects caused by these agents in either group: no inflammation, irritation or allergic reactions of the scalp were observed in any of the subjects; and no subjects complained of itchiness, pain, dryness or scaling of the scalp. One beneficial effect on the scalp was that, according to the dermatologist, 47.4% of the procyanidin B-2 group showed decreased dandruff, whereas only 20% of the placebo group showed this effect (Table 4).

DISCUSSION

Effects on the scalp

Proanthocyanidins are a species of polyphenol known to possess strong antioxidative properties (Hong *et al.*, 1995). It has been reported that proanthocyanidins possess a number of beneficial effects: radical scavenging activity *in vitro* (Vennat *et al.*, 1994), antimutagenic behaviour *in vitro* (Liviero *et al.*, 1994), antitumour-promoting behaviour *in vivo* (Gali *et al.*, 1994), antifungal effects *in vitro* (Eberhardt and Young, 1994), antiviral behaviour both *in vitro* and *in vivo* (Barnard *et al.*, 1993), antidental-plaque formation *in vivo* (Matsudaira *et al.*, 1998), antiulcer effects *in vivo* (Vennat *et al.*, 1989), antiallergic activity *in vitro* (Kanda *et al.*, 1998) and antihypertensive activity *in vivo* (Cheng *et al.*, 1993). Proanthocyanidins have been used as medications aimed at protecting the capillary vessels (Dartenuc *et al.*, 1980); as cosmetics to protect the skin (Wayne, 1996); and as antioxidants in foods and beverages. However, proanthocyanidins in commercial use comprise a mixture of many proanthocyanidin molecules with different degrees of polymerization, which take the form of polymers or oligomers built of various flavan-3-ol units. Procyanidin B-2 can be obtained from the roots of *Fragaria vesca* (Vennat *et al.*, 1988), from the leaves of *Melastoma candidum* (Cheng *et al.*, 1993), or from the inner bark of *Pseudotsuga menziesii* (Douglas fir) (Gali *et al.*, 1994), whereas we obtained highly purified procyanidin B-2 from apple juice (Takahashi *et al.*, 1999a). In this report,

Table 4. Dermatological diagnoses on scalp condition

Group	Number of subjects	Decrease in dandruff	Decrease in sebum secretion	Deleterious effects
Placebo	10	2 (20%)	2 (20%)	0 (0%)
PB2	19	9 (47.4%)	3 (15.8%)	0 (0%)

PB2: procyanidin B-2.

we investigated the effects on the scalp and hair of highly purified procyanidin B-2 from apple juice. Our experimental results indicate that highly purified procyanidin B-2 possesses no irritating side effects: on the contrary, it is highly effective in maintaining the scalp in healthy condition, as evidenced by a decrease in dandruff (Table 4). It is known that the intensity of astringency peaks at the 7-mer of proanthocyanidins (Scholz and Rimpler, 1989). Procyanidin B-2, a dimer of epicatechin, was revealed to possess a low level of astringency and no irritative or inflammatory properties.

Efficacy on hair growth

Male pattern baldness is defined as a hair disease characterized by the miniaturization of terminal hairs (Sullivan and Kossard, 1998) and the vellus transformation of the hair (van Scott and Ekel, 1958). For the evaluation of hair-growing effects, three principal approaches have been reported; these are invasive (biopsies)(Headington, 1984), semi-invasive (epilations)(van Scott *et al.*, 1957; Jackson *et al.*, 1972; Rushton *et al.*, 1983), and non-invasive methods. For non-invasive methods, the phototrichogram technique (Guarrera and Ciulla, 1986; Rushton *et al.*, 1993; Van Neste *et al.*, 1994), the unit area trichogram method (Rushton *et al.*, 1990; Rushton *et al.*, 1991) or the method based on analysis of the diameter of clipped hairs (Tsuji *et al.*, 1994; Ishino *et al.*, 1994) or in weight (Cottington *et al.*, 1977; Price and Menefee, 1990) have been described. In this test, we used the approach of measuring the diameter of clipped hairs and measuring the number of hairs in designated area by the macrophotography method. For the detection of the hair thickening phenomena, the ratio of hairs greater than 40 μm (Rushton *et al.*, 1983) was used as an index. 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 some statistical analyses towards increased hair diameter and density in the procyanidin group results (Tables 2 and 3). According to dermatological diagnosis and subject perception, the efficacy rate of procyanidin B-2 group was higher than that of the placebo, although technical and methodological limitations exist in both indices (Table 3). It is thought that application of procyanidin B-2 causes an increase in the anagen ratio, leading to increased hair diameter and density. With minoxidil, the same effects (Savin, 1987; Roberts, 1987; Kreindler, 1987) or an unsuccessful result (Rushton *et al.*, 1989) have been reported. It has been reported that the anagen ratio undergoes seasonal changes: it rises to a maximum in March and falls to a minimum in September (Randall and Ebling, 1991). Our clinical test was performed from January to May, so the effects of seasonal changes are unlikely to have influenced the overall results.

Conclusions

These results show that the agent comprising procyanidin B-2 exhibits excellent hair-growing effects. The hair diameter and hair density of the subjects in the procyanidin B-2 group were significantly greater than those in the placebo controls (Tables 2 and 3). No deleterious effects on the scalp or skin were seen; on the contrary, procyanidin B-2 treatment improved the scalp, resulting in its attaining a healthier condition; notably, decreased dandruff was observed (Table 4). Further investigations of dose-ranging and long-term experiments are needed to elucidate the pharmacological effects and safety of topical procyanidin B-2 or to investigate the presence of rare adverse effects. We have confirmed from our test that topical procyanidin B-2 is suitable for conducting large-scale clinical trials and have concluded that a large-scale trial would generate useful information on its curative effects on male pattern baldness.

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