Hair growth promoting effects of emodin in telogenic C57BL/6 mice

Jung-Min Yon1, Seul Gi Park1, Chunmei Lin2, Lee Wha Gwon1, Jong-Geol Lee1, Beom Jun Lee1, Young Won Yun1, Sang-Yoon Nam1,*

1College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea
2College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130-118, China
3Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul 05505, Korea

(Received: May 2, 2016; Revised: May 24, 2016; Accepted: May 30, 2016)

Abstract: Emodin is an anthraquinone derivative from the roots of Rheum officinale Baill that possesses a variety of biological activities, including inhibition of 5α-reductase and prostaglandin D2. In this study, we investigated whether emodin promotes hair growth. After emodin was topically applied to the shaved dorsal skin of telogenic C57BL/6N mice, the hair growth rate and morphological analysis were evaluated in dorsal skin for 15 days. After 13 days of treatment, minoxidil or emodin (0.01% or 0.1%)-treated groups showed remarkable regrowth of hairs relative to the vehicle control group. Scoring of the hair growth and rate of hair growth area for 15 days revealed that groups treated with minoxidil and 0.1% emodin were significantly higher than the vehicle control group. Histological examination revealed the emodin and minoxidil groups markedly recovered the number and morphology of hair follicles, including the subcutis depth, relative to the vehicle group. These results suggest that emodin has an excellent promoting effect in hair growth similar to that of minoxidil and might be useful for treatment of baldness or alopecia.

Keywords: C57BL/6 mice, alopecia, emodin, hair growth

Introduction

Hair loss is a common and distressing symptom that affects men and women of all ages. Hair loss, which has a variety of causes including androgenetic alopecia, radiotherapy, chemotherapy, and nutrition, occurs when the normal cycle of hair growth changes. Hair growth is controlled by a unique repetitive cycle comprised of anagen, catagen and telogen phases. Therefore, hair follicles undergo cyclic changes between phases of rapid growth (anagen), regression (catagen), and resting (telogen). Normally, hair follicles are contracted after the anagen phase and the hair shaft falls out during the catagen or telogen phase. As the follicles begin a new anagen phase, it grows back to its original size and produces new hair of normal thickness [13, 18].

Androgenetic alopecia is the most common cause of hair loss affecting both men and women. The 5α-reductase related to androgenetic alopecia is responsible for converting testosterone into dihydrotestosterone (DHT). Because DHT shortens the growth phase of the follicles and size of the hair follicles, it is considered to be a potent agent at triggering hair growth and/or hair loss [7, 8, 21]. Recent studies have reported that prostaglandin D2 (PGD2) was abnormally more abundant in a bald scalp than a haired scalp, especially for patients with androgenetic alopecia, and that it had the capacity to decrease hair lengthening [5, 11].

Emodin (from Rheum emodi, a Himalayan rhubarb), 6-methyl-1,3,8-trihydroxyanthraquinone, which is the most common natural anthraquinone, occurs in the roots and rhizomes of several higher plants, fungi, and lichens [19]. It has been reported that emodin possesses various biological activities, including anti-inflammatory, anti-bacterial, and anti-cancer activities. Emodin also inhibits the 5α-reductase activity and reduces serum PGD2 levels in a dose-dependent manner [3, 9, 17, 20], suggesting that it would be a good candidate for prevention of androgenetic alopecia.

The number of men and women who suffer hair loss and/or hair thinning are increasing in accordance with changes in lifestyle and nutritional balance. Therefore, it is important to develop new materials to prevent hair loss and promote hair growth. However, despite a variety of activities of emodin, the hair growth promoting effects of emodin are currently unknown. In this study, we examined the hair growth promoting effects of emodin in C57BL/6 mice that were synchronized in the telogen stage.
Materials and Methods

Chemicals
Emodin was purchased from Sigma Chemical Co. (St, Louis, MO, USA) and dissolved in 50% ethanol. We used two concentrations of emodin (0.01% and 0.1%) in ethanol. In addition, 5% minoxidil as the positive control was purchased from Hyundai Pharmaceutical (Korea).

Animal experiments
Male C57BL/6 mice (5 weeks old) were purchased from KOATECH (Korea). The animals were housed in polysulphone cages in a well-ventilated room maintained at 21 ± 2°C and 55 ± 10% relative humidity under a 12 hour light/dark cycle. The animals were fed standard mouse chow (Samyang, Korea) and provided tap water ad libitum. All experiments were approved by the Chungbuk National University Animal Care Committee and conducted according to the Guide for Care and Use of Animals (Chungbuk National University Animal Care Committee, CBNUA-469-13-02). To produce new hairs, existing follicles undergo cycles of growth (anagen), regression (catagen) and rest (telogen). The skin of C57BL/6 mice in a resting telogen phase is a pale pink color and skin color of an active hair growth anagen phase becomes dark gray or black. Hair growth stops at catagen phase and the skin transitions are back to the telogen phase, returning to a pale pink color. During anagen phase, hair follicles produce an entire hair shaft from tip to root, but during catagen and telogen phases, the follicles become to regress and lie dormant in a resting phase [1, 10]. After confirming homogeneously-pink skin color in the back skin of C57BL/6 mice in a telogen phase, the dorsal skin hair was shaved in this study. The animals were randomly allotted to the experiment, and then subdivided into 4 groups: 50% ethanol (vehicle control), minoxidil, 0.01% emodin, and 0.1% emodin. Starting the following day (1 day), 200 µL of each sample was topically applied daily for 15 days.

Photographs of hair growth states
At 1, 4, 7, 10, 13, and 15 days after topical application,

Fig. 1. Hair growth promoting effects of emodin. Telogen-matched C57BL/6J mice (6 weeks old) were shaved and topically treated with vehicle (50% ethanol), 5% minoxidil, or emodin (0.01% and 0.1%). The back skins were photographed at 1, 7, 10, 13, and 15 days after depilation under anesthesia with diethyl ether. After 13 days, hair growth in the minoxidil, 0.01% emodin, and 0.1% emodin-treated groups was accelerated relative to the vehicle control group.
photographs of each group of mice were taken while the animals were anesthetized under diethyl ether.

Hair growth scoring
Promotion of hair growth was evaluated by observing the skin color, which is indicative of the telogen to anagen conversion [13]. The hair growth effect was morphometrically calculated using the following hair growth score: score 0, no hair growth; score 1, less than 30% growth; score 2, 30% to less than 50% growth; score 3, 50% to less than 70% growth; score 4, 70% to 100% growth [12]. The rate of hair growth area to clipped area was (hair growth area/clipped area) × 100.

Histological examination
Mice were euthanized with diethyl ether, after which the dorsal skin tissue was extracted. At 7 and 15 days, mice were sacrificed to obtain dermal skin specimens. Skin samples were removed, fixed in 10% neutral buffered formalin and processed according to routine histological techniques. Para-plast-embedded tissues were sectioned to a thickness of 4 µm, then stained with hematoxylin and eosin (H&E).

Statistical analysis
Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Statistical significance was established at \( p < 0.05 \). All data were expressed as the mean ± SE.

Results

Change in hair growth
Emodin application did not show any adverse effects in dorsal dermal skin for the duration of the experiment. The vehicle control group revealed a low recovery in hair growth. However, the skin colors in groups treated with minoxidil, 0.01% emodin, and 0.1% emodin began to change to light gray after 7 days, and on 10 days the skin color had changed from pink to black in all groups of mice. After 13 days, hair growth in the minoxidil, 0.01% emodin, and 0.1% emodin-treated groups was accelerated relative to the vehicle control group (Fig. 1).

Hair growth scores
As shown in Table 1, the dorsal skin of mice was hairless on days 1 to 10 (hair growth score: 0) in all groups. However, at day 13, hair growth was significantly higher in the minoxidil and 0.1% emodin groups than the vehicle group. Hair growth scores in the minoxidil, 0.01% emodin, and 0.1% emodin-treated groups were accelerated relative to the vehicle control group for 15 days (\( p < 0.05 \)).

Rate of hair growth area to clipped area
At day 13, hair growth rates in the minoxidil (57 ± 8.5%) and 0.1% emodin (53 ± 8.9%) groups were significantly higher than in the vehicle group (34 ± 7.3%). At day 15, minoxidil

<table>
<thead>
<tr>
<th>Day</th>
<th>Vehicle</th>
<th>Minoxidil</th>
<th>0.01% emodin</th>
<th>0.1% emodin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>1.5 ± 0.33</td>
<td>2.7 ± 0.39'</td>
<td>2.2 ± 0.59</td>
<td>2.4 ± 0.40'</td>
</tr>
<tr>
<td>15</td>
<td>2.8 ± 0.34</td>
<td>3.8 ± 0.17'</td>
<td>3.3 ± 0.29'</td>
<td>3.6 ± 0.23'</td>
</tr>
</tbody>
</table>

Hair growth scoring index: no hair growth (0), < 25% hair growth (1), 25–50% hair growth (2), 50–75% hair growth (3), and 75–100% hair growth (4). The results shown are the mean values ± SE (\( p < 0.05 \) vs. vehicle).

Histological examination
An increase in the number and size of hair follicles is considered an indicator of the transition of hair growth from the telogen to the anagen phases [16]. An increase of the size and number of hair follicles can be observed in the deep subcutis during the anagen phase [4]. To investigate the progression of hair follicles in the hair cycle, H&E staining was conducted. At day 7, the hair follicles in the 0.1% emodin group had progressed to the anagen stage, whereas those in the other groups remained in the telogen stage. At day 15, the emodin and minoxidil groups showed markedly increases in the number and morphology of hair follicles, including the subcutis depth, relative to the vehicle group (Fig. 3).
Hair growth effect of emodin

Discussion

Hair loss is caused by many factors ranging from genetics to the environment. Recently, the number of men and women suffering from hair loss has increased dramatically. Although hair loss disorder is not life-threatening, it is emotionally distressing to the patient and causes afflicted individuals to have withering during daily social interactions. Therefore, it is of great importance to develop new materials to prevent hair loss and promote hair growth.

Minoxidil is widely used for the treatment of hair loss and is available without a prescription. This compound effectively promotes hair growth by dilating blood vessels and opening potassium channels in both males and females with androgenic alopecia [6, 14]. It is generally well maintained, but side effects including severe allergic reaction, hypertrichosis, and temporary hair loss have been reported [2, 15]. Recently, herbal medicines have received increased attention owing to their hair promoting potential with no side effects. In this study, we examined the hair growth promoting effects of emodin in C57BL/6 mice.

Emodin showed no adverse side effects during 15 days of dermal application to the mice. The color of dermal skin was light gray in the emodin (0.01% and 0.1%) treated groups on day 7 after depilation. At day 13, the emodin (0.01% and 0.1%) groups surpassed vehicle group in hair growth activity, similar to the minoxidil group. Furthermore, the 0.1% emodin group had significantly higher hair growth and rate of hair growth area than the vehicle group after 13 days. In the representative longitudinal sections, increasing hair follicles and a thickened epidermis were observed in the emodin treated groups compared with the vehicle group. These findings indicate that emodin has hair growth promoting activity similar to minoxidil in rodent models.

Fig. 3. Hair follicle growth in telogen-matched C57BL/6J mice treated with emodin. At day 15, the emodin and minoxidil groups showed markedly increased number and morphology of hair follicles including the subcutis depth relative to the vehicle group. The effects of 0.01% and 0.1% emodin on the hair follicles in mice were analyzed using hematoxylin-eosin staining. Longitudinal sections of the back skins (7 and 15 days) were stained, and the image shown is a representative picture of mice. 100×.
Androgenetic alopecia, which is known to be the most common cause of hair loss, is associated with increased DHT levels. Five alpha-reductase, which is produced in many tissues of males and females, participates in steroid metabolism that leads to conversion of testosterone into DHT [3, 7, 21]. PGD2 also inhibits hair growth of patients with androgenetic alopecia and has the capacity to decrease hair lengthening [5, 11]. Emodin possesses antioxidative activities including increased superoxide dismutase (SOD) activity, and SOD1, SOD2, and cytosolic glutathione peroxidase mRNA as well as anti-inflammatory activities. Also, emodin inhibited 5α-reductase by the hydroxyl group and attenuated the PGD2 activities by blocking NF-κB, a major transcription factor for COX-2 in a dose dependent manner [3, 9, 22]. Therefore, based on the results of the present study, we can infer that emodin may act via inhibitions of 5α-reductase and PGD2 activities, as well as through antioxidative and anti-inflammatory activities. In this study, we demonstrated morphologically that two doses of emodin (0.01% or 0.1%) promote the hair growth in dorsal skin of the shaved telogenic mice.

In conclusion, emodin significantly promoted hair growth with no side effects in a mouse model. It is possible that the hair follicles were stimulated to enter into anagen by emodin application, resulting in shortening of the time required for full growth. Although emodin was found to have a definite hair growth promoting effect in this study, further investigations are needed to clarify its molecular mechanism of action.

Acknowledgments

This work was supported by the Basic Science Research Program (2013R1A2A2A03016519 and 2014R1A1A3052735) and the Priority Research Centers Program (2015R1A6A1A04020885) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology and by a research grant from Chungbuk National University in 2013.

References